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Original Article

ANTIGENIC FRACTIONS IN RAGWEED POLLEN

II. FURTHER PURIFICATION OF THE WATER-SOLUBLE FRACTIONS AND A STUDY OF ALKALI-SOLUBLE FRACTIONS AND AN ESTIMATION OF THESE FRACTIONS IN POLLEN EXTRACT

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THIS paper describes further purification of the water-soluble fractions of low ragweed pollen protein previously described,[1] also the separation of water-insoluble, alkali-soluble protein fractions of this pollen, as well as an estimation of the amounts of these fractions in the ragweed pollen extract.

Langner and Kern[2] found no evidence that extracts prepared from pollen ground in a ball mill differed qualitatively from extracts of whole pollen whether the extracts were made in physiologic saline or alkaline extracting fluid. The amount of protein nitrogen was usually greater in the ground ragweed pollen extract. Eagle, Arbesman, and Winkenwerder[3] in a study of ball mill extracts of whole undefatted ragweed pollen and extracts of defatted pollen failed to show any definite immunologic differences, although the ball mill extracts had a higher protein content. In former papers[4, 5] from this clinic it was shown that greater amounts of protein were extracted from pollen by alkaline saline than by distilled water, although direct skin tests on ragweed-sensitive patients indicated that the material obtained in successive alkaline extracts following water extraction showed less skin reactivity.

SUCCESSIVE EXTRACTIONS OF LOW RAGWEED POLLEN AT DIFFERENT HYDROGEN-ION CONCENTRATIONS

Five grams of low ragweed pollen, which had previously been extracted with anhydrous ethyl ether, were successively extracted with

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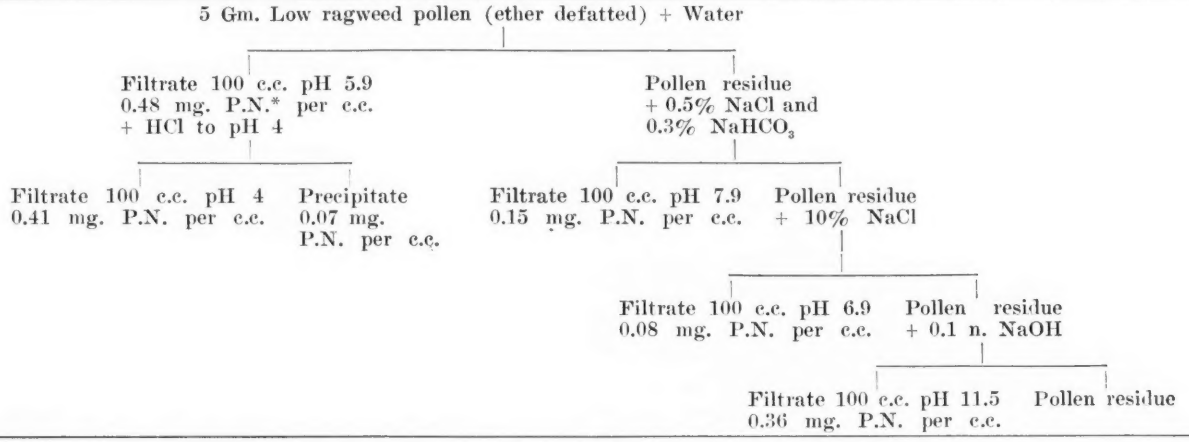
TABLE I

SUCCESSIVE EXTRACTIONS OF LOW RAGWEED POLLEN


5 Gm. Low ragweed pollen (ether defatted) + Water
Filtrate 100 c.c. pH 5.9
0.48 mg. P.N.* per c.c.
+ HCl to pH 4
Pollen residue
+ 0.5% NaCl and
0.3% NaHCO3
Filtrate 100 c.c. pH 4
0.41 mg. P.N. per c.c.
Precipitate
0.07 mg.
P.N. per c.c.
Filtrate 100 c.c. pH 7.9
0.15 mg. P.N. per c.c.
Pollen residue
+ 10% NaCl
Filtrate 100 c.c. pH 6.9
0.08 mg. P.N. per c.c.
Pollen residue
+ 0.1 n. NaOH
Filtrate 100 c.c. pH 11.5
0.36 mg. P.N. per c.c.
Pollen residue

*Protein nitrogen.

100 c.c. portions of distilled water, sodium bicarbonate extracting fluid (0.5 per cent sodium chloride, 0.3 per cent sodium bicarbonate), 10 per cent sodium chloride solution, and 0.1 N. sodium hydroxide solution. The hydrogen-ion concentration was determined on each extract by means of a Beckman pH meter and the amount of nitrogen* by phosphotungstic acid precipitation.[5] The results in Table I show that increased amounts of nitrogen were extracted as the pH of extraction was changed from pH 5.9 to pH 11.5. With 10 per cent sodium chloride little nitrogen was extracted.

PREPARATION OF WATER-SOLUBLE AND ALKALINE-SOLUBLE FRACTIONS

Since preliminary study showed that additional amounts of protein were obtained by alkaline solutions, after water extraction, the following procedure was undertaken to prepare sufficient quantity of these alkali-soluble fractions for chemical and biologic study.

One hundred and fifty grams of defatted low ragweed pollen were extracted with 1,500 c.c. of distilled water, followed by a second extraction with 500 c.c., and the two extracts were combined and designated Extract 1.

The pollen residue was then extracted with two 1,000 c.c. portions of sodium bicarbonate-sodium chloride solution (0.5 per cent NaCl, 0.3 per cent $NaHCO_3$), and these were combined and designated Extract 2.

The pollen residue was further extracted with 1,000 c.c. of 0.01 N. sodium hydroxide and the extract designated Extract 3.

The pH of these extracts and the scheme used is shown in Table II. Each extraction was carried on for fourteen hours at 8° C.

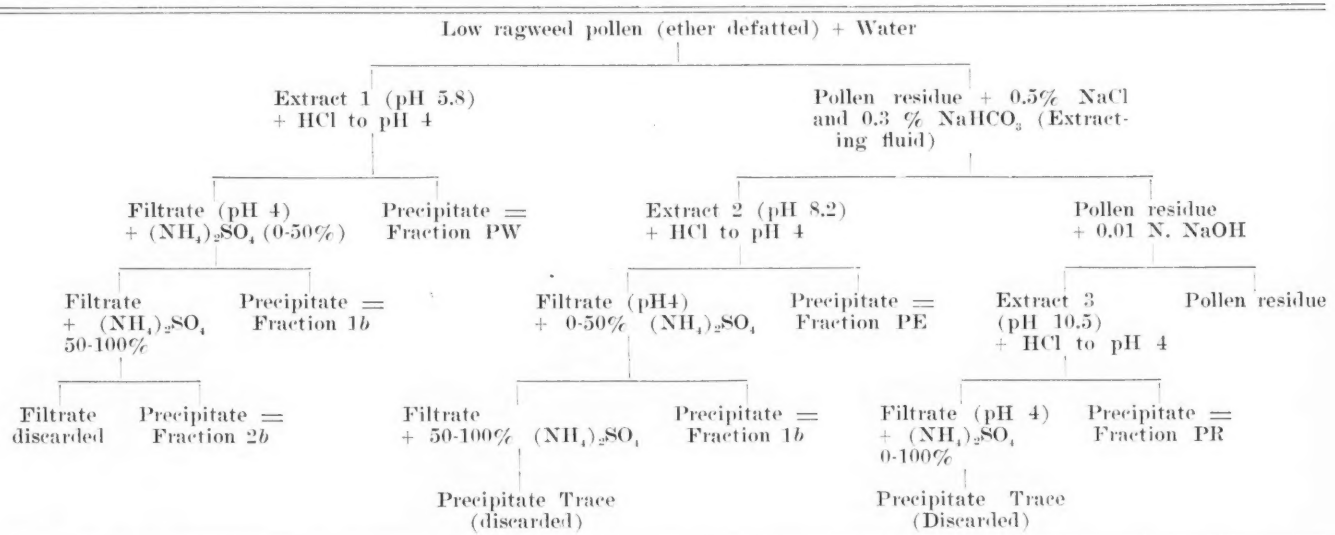
These three extracts were separately changed to pH 4 by the careful addition of hydrochloric acid, when precipitates separated from each extract and were called Fraction PW, Fraction PE, and Fraction PR, respectively. These precipitates were then separately purified by washing with distilled water at pH 4 and by ten successive reprecipitations at pH 4 after solution in 0.01 N. sodium hydroxide. For purposes of subsequent biologic tests these purified fractions were dissolved in 0.01 N. sodium hydroxide, dialyzed against water to remove any excess alkali, Seitz-filtered, and analyzed for nitrogen (P.T.A.). One portion of the solution of Fraction PW was dried in the cryochem apparatus and analyzed (N. 10.11 per cent). Another portion was reprecipitated at pH 4 (fraction PW acid precipitate), washed well with water, dried, and analyzed (N. 11.9 per cent).

The filtrates from Extract 1 and Extract 2, after removal of Fraction PW and Fraction PE, respectively, at pH 4, were brought to 50 per cent saturation with ammonium sulfate and the precipitates were combined

*Nitrogen determined by phosphotungstic acid precipitation hereafter will be designated (P. T. A.).

TABLE II

SCHEME OF SEPARATION OF WATER-SOLUBLE AND ALKALI-SOLUBLE FRACTIONS FROM LOW RAGWEED POLLEN


Low ragweed pollen (ether defatted) + Water
Extract 1 (pH 5.8) + HCl to pH 4
Pollen residue + 0.5% NaCl and 0.3 % NaHCO3 (Extracting fluid)
Filtrate (pH 4) + (NH4)2SO4 (0-50%)
Precipitate = Fraction PW
Extract 2 (pH 8.2) + HCl to pH 4
Pollen residue + 0.01 N. NaOH
Filtrate + (NH4)2SO4 50-100%
Precipitate = Fraction 1b
Filtrate (pH4) + 0-50% (NH4)2SO4
Precipitate = Fraction PE
Extract 3 (pH 10.5) + HCl to pH 4
Pollen residue
Filtrate discarded
Precipitate = Fraction 2b
Filtrate + 50-100% (NH4)2SO4
Precipitate = Fraction 1b
Filtrate (pH 4) + (NH4)2SO4 0-100%
Precipitate = Fraction PR
Precipitate Trace (discarded)
Precipitate Trace (Discarded)

and designated Fraction 1*b*. The material precipitating from 50 to 100 per cent saturation with ammonium sulfate from Extract 1 filtrate was designated Fraction 2*b*.

When ammonium sulfate was added to saturation to Extract 2 filtrate and to Extract 3 filtrate, only traces of precipitate resulted which were discarded.

Fraction 1*b* was reprecipitated at 0 to 50 per cent saturation with ammonium sulfate at pH 4 until completely water soluble and practically colorless. Fraction 2*b* was reprecipitated at 50 to 100 per cent ammonium sulfate saturation until free from water-insoluble material, dialized, and then redissolved at pH 4. From one portion the material precipitating from 60 to 90 per cent saturation with ammonium sulfate was designated Fraction 2*b*. Fraction 2*c* from another portion was precipitated from 50 to 66 per cent saturation, and Fraction 2*d*, from a third portion, was precipitated at 66 to 100 per cent saturation with ammonium sulfate. These fractions were then dialyzed against distilled water until free of sulfate ion, portions were dried in the cryochem apparatus for analyses, and to other portions 0.5 per cent sodium chloride and 0.3 per cent sodium bicarbonate were added. They were Seitz-filtered, analyzed for nitrogen (P.T.A.), and used for chemical and biologic tests.

In another preparation of fractions from a water extract of 200 Gm. of low ragweed pollen, Fraction 1 was divided into two parts; Fraction 1*c* was precipitated by ammonium sulfate in a concentration up to 28 per cent and Fraction 1*d* from 28 to 50 per cent saturation at pH 4. The further addition of ammonium sulfate to 58 per cent precipitated material which proved to be a mixture of Fraction 1 and Fraction 2 and was therefore discarded. Fraction 2 was also divided into Fraction 2*e*, precipitating from 58 to 66 per cent saturation, and Fraction 2*f*, from 66 to 90 per cent, and since only traces of protein were precipitated from 90 to 100 per cent saturation with ammonium sulfate, this latter was discarded.

The initial protein precipitate which separated from the water extract on changing the pH to 4 in this particular study has been designated Fraction PW_1 and two subsequent protein precipitates which failed to dissolve in water appearing during the purification of Fraction 1 were called Fraction PW_2 and Fraction PW_3. These three fractions were purified separately by solution in 0.01 N. sodium hydroxide and by reprecipitation at pH 4. A portion of Fraction PW_1 (acid precipitate) was dried for analysis. The remaining solution as well as solutions of Fractions PW_2 and PW_3 were prepared for chemical and biologic tests as previously described for the solution of Fraction PW.

Solubility tests on Fraction PW_1 were carried out as follows: Ten cubic centimeters of the purified solution containing 0.85 mg. protein

TABLE III

CHEMICAL TESTS AND ANALYSES ON THE FRACTIONS FROM LOW RAGWEED POLLEN

PREPARATION OF LOW RAGWEED FRACTIONS	ANALYSES DRIED TO CONSTANT WEIGHT PER CENT				TESTS ON SOLUTIONS								
	ASH	NITROGEN	NITROGEN (ASH-FREE)	PHOSPHORUS	COLOR	REDUCTION BENEDICT'S AFTER HYDROLYSIS	HEATING WITH 5 PER CENT TRICHLORACETIC ACID	CONCENTRATED HNO_3 + HEAT	BIURET TEST	SAT. Na_2SO_4	TRYPTOPHANE TEST	PICRIC ACID	P.T.A.†
Fraction 1*	4.53	11.30	11.83	Negative	None	Negative	Precipitate	White precipitate turns pale yellow	Pink-purple	Precipitate	Deep blue	Precipitate	Quantitatively precipitated
Fraction 1*b*	2.26	13.24	13.54	Negative	None	Negative	Precipitate	White precipitate turns pale yellow	Pink-purple	Precipitate	Deep blue	Precipitate	Quantitatively precipitated
Fraction 1*c*					None	Negative	Precipitate	White precipitate turns pale yellow	Pink-purple		Deep blue	Precipitate	Quantitatively precipitated
Fraction 1*d*					None	Negative	Precipitate	White precipitate turns pale yellow	Pink-purple	Precipitate	Deep blue	Precipitate	Quantitatively precipitated
Fraction 2*	5.3	1.68	1.75	Negative	Brown	Positive	No precipitate	Negative	Pink-purple	No precipitate	Faint blue	No precipitate	Quantitatively precipitated
Fraction 2*b*	2.05	8.11	8.28	Negative	Light brown	Negative	No precipitate	Negative	Pink-purple				Quantitatively precipitated
Fraction 2*c*	5.24	11.45	12.08		Light brown	Negative	Trace	Negative	Pink-purple	No precipitate	Faint blue	No precipitate	Quantitatively precipitated

Fraction 2*c*	5.24	11.45	12.08		Light brown	Negative	Trace	Negative	Pink-purple	No precipitate	Faint blue	No precipitate	Quantitatively precipitated
Fraction 2*d*	1.04	3.16	3.29		None	±	No precipitate	Negative	Pink-purple				Quantitatively precipitated
Fraction 2*e*	1.15	9.92	10.03		None	Negative	No precipitate	Negative	Pink-purple		Very faint blue		Quantitatively precipitated
Fraction 2*f*	0.55	9.41	9.46		None	±	No precipitate	Negative	Pink-purple	No precipitate	Very faint blue	No precipitate	Quantitatively precipitated
Fraction 3PW	4.05	10.11 11.9 acid precipitate	10.54	Negative	Brown	Negative	Precipitate	White precipitate turns pale yellow	Violet-purple	Precipitate	Faint blue	Precipitate	Quantitatively precipitated
Fraction 3PE					Brown	Negative	Precipitate	White precipitate turns pale yellow	Violet-purple		Faint blue	Precipitate	Quantitatively precipitated
Fraction 3PR					Brown	Negative	Precipitate	White precipitate turns pale yellow	Violet-purple		Faint blue	Precipitate	Quantitatively precipitated
Fraction $3PW_1$		13.08 acid precipitate			Brown	Negative	Precipitate	White precipitate turns pale yellow	Violet-purple		Faint blue	Precipitate	Quantitatively precipitated
Fraction $3PW_2$					Brown	Negative	Precipitate	White precipitate turns pale yellow	Violet-purple		Faint blue	Precipitate	Quantitatively precipitated
Fraction $3PW_3$					Brown	Negative	Precipitate	White precipitate turns pale yellow	Violet-purple		±	Precipitate	Quantitatively precipitated

*Preparations described in previous paper.[1]
†Phosphotungstic acid.

nitrogen (P.T.A.) per cubic centimeter (and no nonprotein nitrogen) were acidified to pH 4 and after standing for twelve hours at 7° C. the precipitate PW_1 was removed by the centrifuge and nitrogen was determined on the supernatant. It contained 0.10 mg. nitrogen per cubic centimeter. The fact that Fraction PW_1 was not completely insoluble at pH 4 explained the subsequent separation of Fractions PW_2 and PW_3.

COMPARATIVE ANALYSES AND CHEMICAL TESTS ON THE SEPARATED FRACTIONS

The analyses of the dry material from these low ragweed fractions and certain characteristic tests on their solutions are given in Table III. Not all tests were done on each preparation.

The preparations of Fraction 1, Fraction 1*b*, Fraction 1*c*, and Fraction 1*d* were alike chemically as shown in Table III. Two preparations on which analyses were carried out (Fraction 1 and Fraction 1*b*) contained 11.83 and 13.54 per cent nitrogen. Chemically these preparations resembled vegetable albumin.

The nitrogen content of the six preparations of Fraction 2 varied from 1.7 per cent to 12.08 per cent, an indication that protein was only a portion of certain of these preparations. Chemical tests on these preparations are listed in Table III and show the differences between Fraction 1 and Fraction 2. Three preparations (Fraction 2, Fraction 2*d* and Fraction 2*f*) reduced Benedict's solution, indicating presence of carbohydrate, while three others (Fraction 2*b*, Fraction 2*c*, and Fraction 2*e*) did not. Only one preparation (Fraction 2*c*) gave slight precipitation on heating with trichloracetic acid, indicating contamination with Fraction 1, confirmed by biologic test. Fraction 2 preparations in solubility and by their failure to precipitate on heating were similar to vegetable proteoses which Wells and Osborne[6] showed were highly anaphylactogenic.

The various preparations designated as Fraction PW, Fraction PE, and Fraction PR were alike in their chemical reactions, and analyses of two different preparations, Fraction PW (acid precipitate) derived from one lot of pollen and Fraction PW_1 (acid precipitate) from another lot of low ragweed pollen, were typical of protein. The material recovered from their solution in dilute alkali by drying in the cryochem apparatus was slightly lower in nitrogen, possibly due to variation in the inorganic ash content which was not determined on all samples. All these preparations were relatively insoluble in water; they precipitated at a pH of 4, dissolved at a pH below 2.5 and at slightly alkaline hydrogen-ion concentrations. These fractions showed marked differences in solubility from Fraction 1 and Fraction 2. They all precipitated with picric acid, trichloracetic acid, phosphotungstic acid, and with saturated sodium sulfate. They gave only a faint positive test for

tryptophane. One preparation, Fraction PW_3, which subsequent biologic tests showed was the purest preparation and relatively free of Fraction 1, gave a doubtful positive test for tryptophane. They all gave a violet-purple color in the biuret test. These alkali-soluble proteins resembled plant glutelins and are all now designated as Fraction 3.

No detectable amounts of Fraction 1 or Fraction 3 passed through ordinary Visking membranes, but Fraction 2 did dialyze slowly, an indication of its smaller molecular size. Fraction 1 and Fraction 2 filtered easily through Seitz filters but Fraction 3 filtered with difficulty, a characteristic of plant glutelins.

COMPARATIVE TESTS OF ALLERGIC ACTIVITY

The low ragweed fractions were diluted on the protein nitrogen unit basis (1 unit = 0.00001 mg. protein nitrogen) (P.T.A.) for comparison in various biologic tests.

Direct skin tests on typical untreated cases of ragweed hay fever by the intracutaneous injection of 0.01 to 0.02 c.c. of these fractions showed definite differences in activity. The relative activity of the various fractions was different in different sensitive individuals, indicating that there was more than one active antigen in the pollen. However, these differences were more clearly shown by the indirect method of testing (passive transfer). For this method triplicate skin sites were prepared in the backs of normal test subjects by the intracutaneous injection of 0.05 c.c. of sera taken from patients with ragweed hay fever who had never received injections of ragweed extract. These sites were tested with 0.025 c.c. of 1 unit dilutions of Fraction 1, Fraction 2, and Fraction 3 (PW_3) and the reactions recorded. When sera from fifty-two cases were so tested, fifty (96 per cent) gave positive tests with a solution of Fraction 1 containing 1 unit per cubic centimeter, sixteen (30 per cent) also reacted to Fraction 2, and ten (19 per cent) gave reactions to Fraction PW in the same dilution. A typical protocol of tests on sites prepared in one test subject from thirteen sera is given in Table IV.

The relative activity of various preparations of fractions was determined by a semiquantitative dilution method. A number of skin sites were made with a 1:10 dilution of the serum under study in the same test subject. These sites were tested with serial dilutions (differing tenfold in protein nitrogen concentrations) of the preparations studied. The relative activity was indicated by the highest dilution just giving a positive reaction. This method is illustrated in Table V, where different preparations of Fraction 3 are compared in particular.

When new preparations of Fraction 1 were made, they were compared with the previous ones by the method just described. Such various

TABLE IV

REACTIONS OF RAGWEED FRACTIONS (1 UNIT PER CUBIC CENTIMETER) ON PASSIVELY SENSITIZED SKIN SITES

RAGWEED-SENSITIVE SERA	REACTION OF TRIPLICATE SITES ON TEST WITH		
	FRACTION 1	FRACTION 2	FRACTION 3 (PW_3)
Etn	+++	±	0
Eta	++++	+++	+++
Fiw	+++	0	0
Gro	++	++	0
Gug	++	++	+
Her	++	±	0
Hih	+++	0	0
Jon	+++	0	0
Kit	+++	0	0
Kln	+++	0	0
Ken	+++	0	0
Klr	+++	0	0
Lip	+++	++	++

TABLE V

COMPARATIVE REACTIVITY OF PROTEIN FRACTIONS ON SKIN SITES PASSIVELY SENSITIZED WITH RAGWEED-SENSITIVE SERA (DILUTED 1:10)

LOW RAGWEED FRACTIONS	UNITS PER C.C.					UNITS PER C.C.				
	10	1	0.1	0.01	0.001	10	1	0.1	0.01	0.001
	Kug Serum					*Lip Serum*				
Fraction 1*c*		++	±	0			+	±		
Fraction PW		+	0				+	+	0	
Fraction PR	+	0				+	0			
Fraction PE		+	0	0			+	0		
Fraction PW_1		+	0				±	±	0	
Fraction PW_2		+	±	0			+	0		
Fraction PW_3		+	0				+	0		
	Etn Serum					*Adr Serum*				
Fraction 1*c*	+	±	0				+	±	0	
Fraction PW	±	0				+	±	0	0	
Fraction PR	+	0				+	0			
Fraction PE		+	0				+	0		
Fraction PW_1	±	0				±	0			
Fraction PW_2	+	0					+	0		
	Gro Serum					*Tke Serum*				
Fraction 1*c*		++		++	+		++		++	±
Fraction PW		+	0				±	0		
Fraction PW_1		+	0			±	±	0		
Fraction PW_3	++	±				+	0			
Fraction PE		++	±	0		+	0			
Fraction PR	+	0				+	0			
	Cos Serum					*Wol Serum*				
Fraction 1*c*	±	0					+++		++	±
Fraction 2*f*		+	±	±	0		+		±	±
Fraction PW_3	±	±	±				+++		++	±
Fraction LR (Whole extract)	+	±	0							

preparations reacted with each serum in the same dilution range within the experimental error.

Fraction 1*c*, precipitated from 0 to 28 per cent saturation, and Fraction 1*d*, precipitated from 28 to 50 per cent saturation with ammonium

sulfate, and prepared at the same time, were compared by the semi-quantitative dilution method and also in the neutralization of sera by passive transfer.[1] They were alike in activity by both tests, indicating that after removal of alkali-soluble material all the fractions precipitated to 50 per cent saturation with ammonium sulfate seemed to be a single protein substance.

The solid material from the six different preparations of Fraction 2 contained from 1.75 to 12.08 per cent nitrogen. Certain ones contained carbohydrate while others did not. However, when these preparations were tested in dilutions containing the same amount of protein nitrogen on sites made with sera (sensitive to Fraction 2) in a 1:10 dilution in the backs of test subjects, they showed comparable activity irrespective of whether carbohydrate was present or absent. This indicated that the activity was associated with the protein nitrogen and that the carbohydrate was an inactive contaminant.

Sera giving positive reactions to 1 unit dilutions of Fraction PW were used (in a 1:10 dilution) for making sites on which to test the alkali-soluble fractions. When the different alkali-soluble fractions were tested on these sites, comparable skin reactions resulted with the different preparations at the same protein nitrogen dilution. In Table V are given representative examples of some of these tests. Although some of these preparations, such as Fraction PE and Fraction PR, had been extracted from the pollen after the removal of Fraction PW and at a higher alkalinity, they were alike in chemical properties as well as in allergic activity. Since the work so far suggests these fractions are identical, as previously stated, they have been designated Fraction 3. However, only eight suitable sera were available for comparing these preparations, and there is a possibility that further study might disclose sera reacting differently to these various preparations.

The previous paper[1] showed cross neutralization tests of various sera with Fraction 1 and Fraction 2, and, since the subsequent work has supported the published results, further details are not given. In Table VI are given cross neutralization experiments with Fraction 3 (PW_3) carried out as described in the previous paper.[1]

Neutralization of sensitive sera with the preparations of Fraction 3 is complicated by the fact that all these preparations are relatively inactive in the neutralization of sera, while Fraction 1 is approximately a hundredfold more active in neutralization with most sera. Therefore, contamination with a small amount of Fraction 1 would seriously interfere with the results. Preliminary studies, using sera reactive with Fraction 1 but not to (so-called) Fraction 3 indicated that Fraction PW_3 contained no appreciable amount of neutralizing activity against Fraction 1. Therefore, it was considered the purest of the preparations of Fraction 3 and the most suitable for neutralization studies. Fraction

PW, Fraction PW_1, and Fraction PW_3 neutralized to test with Fraction PW_3, but Fraction PW_1 and Fraction PW, when mixed with sera in 10,000 unit strength, neutralized somewhat to Fraction 1 as well as to whole extract, an indication of contamination with Fraction 1.

TABLE VI

COMPARATIVE NEUTRALIZATION OF SENSITIVE SERA WITH LOW RAGWEED FRACTIONS

SERUM	MIXED WITH	PROTEIN NITROGEN UNITS	DUPLICATE SITES REACTION ON TEST WITH 1,000 UNITS PER C.C.	
			FRACTION 1	FRACTION 3 (PW_3)
Kug	Fraction PW_3	1000	++++	+
	Fraction PW_3	10,000	++++	±
	Fraction 1*c*	100	+++	+
	Fraction 1*c*	1000	+	+
	Saline		++++	+++
Tke	Fraction 1*c*	1000	0	0
	Fraction PW_3	1000	+++	+
	Fraction PW_3	10,000	+++	0
	Saline		+++	++
Tke	Fraction PW_3	1000	+++	+++
	Fraction PW_3	10,000	+++	0
	Fraction PW_3*	1000	+++	+++
	Fraction PW_3*	10,000	+++	0
	Saline		+++	+++

			QUADRUPLICATE SITES			
			FRACTION 1	FRACTION 2	FRACTION 3(PW_3)	WHOLE EXTRACT
Wol 1:10	Fraction 1*c*	100	0	±	0	0
	Fraction 2*f*	100	+++	+	±	+++
	Fraction 2*f*	1000	0	0	±	0
	Fraction PW_3	100	+++	+	±	+++
	Fraction PW_3	1000	+++	+	0	+++
	Whole extract	100	0	0	0	0
	Saline		+++	+	+	+++

*Extract heated to 70° C. for one hour.

Fraction PW_3 when mixed with a 1:10 dilution of Woh. serum neutralized to test with itself between 100 and 1,000 units, although it failed to neutralize to Fraction 1 or Fraction 2 or whole extract at 1,000 units. Fraction 1 and whole extract in 100 units neutralized to Fraction 1, Fraction PW_3, and whole extract. With Tke. serum, 1,000 units of Fraction 1 neutralized to both Fraction 1 and Fraction PW_3, but Fraction PW_3 did not neutralize to Fraction 1 even in 10,000 unit strength. Heating fraction PW_3 at 70° C. for an hour did not affect its neutralizing capacity for Fraction PW_3 at 10,000 unit strength. Similar heat treatment of Fraction 1 practically destroyed its neutralizing activity for sensitive serum as well as for its direct skin test activity.[1] Fraction 2 seemed to have its serum neutralizing activity reduced by heat, but its skin reactivity on direct test remained essentially the same as well as its desensitizing activity in the Dale test.[1] Fraction 2 and Fraction PW_3 both required much higher concentrations for the neutralization of sera than Fraction 1.

ANTIGENIC STUDIES BY THE DALE TEST

Virgin guinea pigs were sensitized by a single abdominal injection of 50,000 units alum precipitated Fraction PW, Fraction PR, and whole low ragweed extract. After an interval of over fourteen days, the Dale test was performed on the isolated uterine horns as previously described.[1] The antigens (10,000 protein nitrogen units contained in 1 c.c.) were added to the 150 c.c. bath.

In tests on thirteen animals sensitized to whole extract, Fraction 1 and Fraction 2, when added to the Dale bath, desensitized to whole extract. Since 2,000 to 3,000 units of Fraction 3 were contained in the whole extract added, these animals may be considered to show no specific sensitization to Fraction 3.

Three additional pigs sensitized to whole extract and found sensitive to Fraction 1 and Fraction 2 showed no sensitivity to subsequent test with Fraction PW (Fraction 3).

In tests on three pigs injected with Fraction PW (Fraction 3), the uterine horns failed to react when Fraction PW was added. Two of the animals were not sensitized to any of the ragweed preparations tested, but one pig was sensitive to Fraction 1.

Three pigs injected with Fraction PR (Fraction 3) were not sensitized to this fraction, one pig was not sensitized to any of the ragweed preparations tested, one pig showed sensitivity only to Fraction 1, and the other pig was sensitive only to Fraction 2.

For brevity the Dale tests on animals injected with Fraction PW (Fraction 3) and Fraction PR (Fraction 3) are summarized in Table VII and only one representative test on the sixteen animals injected with whole extract is included. The subsequent additions of antigens to prove desensitization and the histamine contractions are not indicated.

Certain guinea pigs injected with preparations of Fraction 3 (Fraction PW and Fraction PR) showed sensitivity to Fraction 1 or Fraction 2 but not to test with the injected Fraction 3 preparation. This indicated that these preparations contained sufficient Fraction 1 or Fraction 2 to sensitize but did not contain sufficient of these impurities (Fraction 1 or Fraction 2) to cause contraction when added to the Dale bath. In this study in the animals injected, no specific sensitization resulted to the alkali-soluble protein designated as Fraction 3, but as previously shown,[1] Fraction 1 and Fraction 2 were anaphylactively active. It seems possible that sensitization of a large series of animals might yield specific sensitivity to Fraction 3, but the present data suggest that this fraction is relatively less antigenic than Fraction 1 or Fraction 2. It is interesting to note, as previously mentioned, that in the study of fifty-two cases of ragweed hay fever, only ten (19 per cent) showed sensitivity to Fraction 3. Therefore it was a less active antigen in man as well as in the experimental animal.

TABLE VII

SUMMARY OF DALE TESTS

PIG NO.	SENSITIZING ANTIGEN	HORN NUMBER	PREPARATION ADDED	CONTRACTION
1	Fraction PW	1	Fraction PW	0
			Whole extract	+
		2	Fraction 2	0
			Fraction 1	+
			Whole extract	0
2, 3	Fraction PW		Fraction PW	Not sensitive
			Fraction 1	Not sensitive
			Fraction 2	Not sensitive
			Whole extract	Not sensitive
4	Fraction PR	1	Whole extract	+
			Fraction PR	0
		2	Fraction 2	+
			Fraction 1	0
			Fraction PR	0
5	Fraction PR	1	Fraction PR	0
			Fraction 2	0
			Fraction 1	+
			Whole extract	0
		2	Fraction PR	0
			Fraction 2	0
			Fraction 1	+
			Whole extract	0
6	Fraction PR		Fraction PR	Not sensitive
			Fraction 1	Not sensitive
			Fraction 2	Not sensitive
			Whole extract	Not sensitive
7*	Whole extract	1	Fraction PW	0
			Fraction 2	+
			Fraction 1	+
			Whole extract	0
		2	Fraction PW	0
			Fraction 2	+
			Fraction 1	+
			Whole extract	0

*Representative of tests on sixteen pigs sensitized to whole extract.

ESTIMATION OF FRACTION 1, FRACTION 2, AND FRACTION 3 IN WHOLE EXTRACT OF RAGWEED POLLEN

Preliminary experiments showed that excess phosphotungstic acid in the presence of 10 per cent hydrochloric acid quantitatively precipitated the nitrogen of each of the three fractions from their purified solutions. Trichloracetic acid precipitated Fraction 1 quantitatively from purified solutions of Fraction 1 but did not precipitate Fraction 2. Fraction 3 was precipitated from the whole pollen extract at pH 4. These differences in solubility made possible an approximate estimation of these fractions in any given extract of defatted ragweed pollen by the following procedure.

The total protein nitrogen of a pollen extract was determined by phosphotungstic acid precipitation as previously described[5]; then the

solution was changed to pH 4 by the addition of hydrochloric acid, using care not to run below that point as greater acidity precipitates Fraction 1, and redissolves Fraction 3. After standing at 7° C. for two hours, the precipitated Fraction 3 was removed by the centrifuge and the protein nitrogen of Fraction 1 plus Fraction 2 was determined by phosphotungstic acid precipitation on one portion of the supernatant. The difference between this and the whole extract = Fraction 3. Another portion of the supernatant was heated with an equal volume of 10 per cent trichloracetic acid in a boiling water bath for one hour, and the precipitated Fraction 1 was filtered off through a small Whatman No. 5 paper and the paper washed with 5 per cent trichloracetic acid. The protein nitrogen of Fraction 2 was then determined on the filtrate and washings with phosphotungstic acid. All determinations were carried out in duplicate. The difference between the protein nitrogen of Fraction 2 and of Fraction 1 plus Fraction 2 gave Fraction 1.

In Table VIII are given some typical analyses. One extract was divided into four parts; one part was analyzed immediately, another after a two-week interval, and the other portions after differing filtration conditions as indicated. Less Fraction 3 was present after a two-week interval at 7° C. than on the day of preparation, but no significant changes had resulted in Fraction 1 or Fraction 2. An extract of the same lot of low ragweed pollen in which 10 Gm. per 100 c.c. were used instead of 5 Gm. gave an extract with approximately double the amounts of the three fractions. Previous work[5] had shown, and it was again confirmed that extracts made from different lots of pollen under identical conditions varied in the amount of protein nitrogen extracted. In the last two columns in Table VIII, extracts from two different lots of pollen show a wide variation in the amounts of these fractions. Pollen K_2 gave an extract with equal amounts of Fraction 1 and Fraction 2 and pollen H_2 extract contained twice as much Fraction 2 as Fraction 1. In five of eight analyses of extracts from different lots of pollen Fraction 1 was approximately one-half as much as Fraction 2. Most extracts contained approximately one-fifth of Fraction 3, but any variation in the pH of extracts altered amounts of this fraction since it was more soluble in alkaline solutions. Extracts made from 5 Gm. of pollen in 100 c.c. of a solution containing 0.5 per cent sodium chloride, 0.3 per cent sodium bicarbonate, and 0.4 per cent phenol had a pH of approximately 7.2 and contained all three fractions.

The effect of incubation for fourteen days at 37° C. on a low ragweed extract is also shown in Table VIII. The fresh extract was passed through a Seitz filter, and after tests had shown that the extract was sterile, it was divided, one part analyzed at once and the other part placed in the incubator for fourteen days at 37° C. and then analyzed. The precipitate which resulted during the incubation was removed by the centrifuge, and the analysis was carried out on the clear supernatant.

TABLE VIII

ESTIMATION OF PROTEIN FRACTIONS IN RAGWEED EXTRACT

POLLEN GM. PER 100 C.C. IN 0.5% NaCl AND 0.3% $NaHCO_3$	5 GM. L. RAGWEED K*				10 GM. L. RAGWEED K*	10 GM. G. RAGWEED H*	5 GM. L. RAGWEED K_1*		5 GM. L. RAGWEED H_1*	5 GM. L. RAGWEED H_2*	5 GM. L. RAGWEED K_2*
Type filtration	PAPER†	PAPER + SEITZ	PAPER + SEITZ	PAPER + 2 TIMES SEITZ	PAPER	PAPER	PAPER + SEITZ		PAPER + SEITZ	PAPER + SEITZ	PAPER + SEITZ
Date extracted	10/27/41				9/16/41	9/22/41	10/14/41		2/18/36	1/8/42	9/25/41
Date analyzed	10/28/41		11/5/41	11/5/41	9/18/41	9/24/41	10/23	11/6‡	2/27/42	1/19/42	10/ 7/41
pH	7.35	7.35	7.4	7.60	6.9	7.0	7.4	7.2	7.2	7.2	7.2
Fraction 1 + Fraction 2 + Fraction 3 (whole extract) P.N. mg. per c.c. (P.T.A.)§	0.47	0.48	0.43	0.43	0.95	0.82	0.44	0.29	0.19‖	0.32	0.55
Fraction 1 + Fraction 2 Filtrate at pH 4 P.N. mg. per c.c. (P.T.A.)§	0.37	0.37	0.36	0.36	0.80	0.74	0.30	0.25	0.15	0.29	0.46
Fraction 2 P.N. mg. per c.c. (P.T.A.)§	0.26	0.25	0.22	0.22	0.55	0.51	0.17	0.18	0.09	0.21	0.23
Fraction 3 (by difference) P.N. mg. per c.c.	0.10	0.11	0.07	0.07	0.15	0.08	0.14	0.04	0.04	0.03	0.09
Fraction 1 (by difference) P.N. mg. per c.c.	0.11	0.12	0.14	0.14	0.25	0.23	0.13	0.07	0.06	0.08	0.23

*Letters refer to lot and source of pollen.
†No. 5 Whatman filter paper used throughout.
‡Fourteen days at 37° C.
§Nitrogen precipitated by phosphotungstic acid.
‖Contained 0.40 mg. protein nitrogen per cubic centimeter when prepared in 1936.

Fraction 1 and Fraction 3 were much less after incubation, although Fraction 2 remained the same. Analyses on four different extracts of from 4 to 6 years in age have shown greatly reduced amounts of all three fractions. A representative analysis of a 1936 low ragweed extract is shown in Table VIII.

These determinations are subject to a certain degree of error due to absorption of one protein by another when precipitated from a mixture and by the fact that Fraction 3 was not completely insoluble at pH 4. The determination of Fraction 2 from fresh and particularly from old extracts would include not only protein but any protein-split products or nitrogenous material precipitated by phosphotungstic acid. ?

SUMMARY

1. Various alkali-soluble protein fractions separated from low ragweed pollen differed chemically and immunologically from Fraction 1 and Fraction 2. They resembled each other chemically and gave positive skin tests on certain ragweed sensitive cases and on skin sites passively sensitized with the sera of certain ragweed-sensitive patients (passive transfer). Therefore, they have been designated Fraction 3.

2. When diluted to contain the same protein nitrogen content, different preparations of Fraction 1 were alike in allergic activity. Similarly the different preparations of Fraction 2 or the different preparations designated Fraction 3 when compared on their protein nitrogen content were of equal allergic activity.

3. The three fractions showed marked differences in their capacity to neutralize ragweed-sensitive sera and their relative activity varied with different sera.

4. Dale tests on sixteen animals sensitized to whole low ragweed extract and on six animals sensitized with preparation of Fraction 3 failed to show sensitivity to Fraction 3 although many sensitizations to Fraction 1 and Fraction 2 resulted.

5. A method is given for the estimation of these three fractions in ragweed extract. In a typical extract of low ragweed pollen, Fraction 1 and Fraction 3 were each approximately one-fourth of the total protein nitrogen while Fraction 2 was approximately one-half.

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ABSORPTION OF ALLERGENS

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THE study which I am about to present is a continuation of a series of investigations which were started in 1926 and have been published under the major heading, The Absorption of Unaltered Protein. The early objections to this title were prompted by a reluctance to believe that protein could be absorbed in an unaltered form from the digestive tract. Sufficient confirmatory evidence has accumulated, however, to eliminate almost all objections on this score. More recently there has been some criticism that the title is misleading because only traces of unaltered protein are absorbed. Nevertheless, such amounts are of fundamental importance in the field of hypersensitiveness, because, under favorable circumstances, they are sufficient not only to sensitize but also to precipitate allergic reactions. Because these minute traces of antigens are of the utmost importance in allergy, this discussion has been given the above title, although in the last analysis it still deals with the absorption of unaltered proteins.

Ingestants and inhalants cause many allergic reactions in organs distant from the alimentary and respiratory tracts. In such instances, the allergen reaches the shock organs by way of the circulation. It therefore becomes important to understand the factors which influence the passage of allergens through the various portals of entry into the body. This information not only helps to clarify our understanding of the mechanisms involved but may also suggest a solution to some of the therapeutic problems in allergy.

In the pioneer experiments of Schloss, Van Alstyne, Wells and others, the immunologic procedures for detecting the absorption of antigen usually depended upon precipitin studies or anaphylactic experiments in animals. In our own studies a direct immunologic method was employed for the immediate detection of the entrance of antigen into the circulation. This was accomplished through the use of a passively sensitized cutaneous site which reacted when the related allergen was absorbed from the site of administration and entered the blood stream.

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In the early studies employing this technique, my colleagues, Brunner, Sussman and Davidson, showed that uncooked fish and egg, taken in ordinary amounts on an empty stomach, appeared in the blood stream within from five to sixty minutes in about 85 per cent of normal adults. Wilson's findings in the study of egg absorption in infants and children up to 4 years of age were similar to those obtained in adults. A series of comparative studies by Gray revealed that, in cases of gastric anacidity, the absorption of peanut allergen was very rapid and occurred at approximately the same rates following oral, intraduodenal, and rectal administrations of the antigen. As the degree of gastric acidity increased, the absorption by all three routes became slower.

We are indebted to the late Henry Straus for demonstrating that the direct technique for studying absorption of allergens could be successfully applied in the rhesus monkey. In studies on this animal, Straus, Harten, Gray, and Livingston succeeded in demonstrating that the introduction of a few milliliters of cottonseed "milk" into the stomach or esophagus, after both ends of these organs had been cut and clamped, resulted in absorption of the allergen in from two to eleven minutes. The long-debated question as to whether allergenic absorption occurs from the stomach itself was thus answered in the affirmative. The same workers also reported the absorption of cottonseed within five minutes after its injection into gall bladders of which either the common duct or the cystic duct had been clamped.

In human beings the antigen tide in the circulation, following the ingestion of an allergen, was studied by A. Walzer, with the "reverse" technique. It was found that the antigen tide, after a peanut meal, rose sharply in the blood for about one hour. Then came a gradual fall of the tide for about eight hours, followed by an even slower decline, until no trace of the antigen remained at forty-eight hours. With the reverse technique it was also shown that small amounts of peptone, dilute hydrochloric acid, or mineral oil, when administered before the antigen meal, were of therapeutic value in that they diminished the antigen tide, while spiritus frumenti, similarly administered, was deleterious in that it accelerated and increased absorption.

Rosenzweig studied the absorption of peanut antigen from the human vagina and uterine cervix. Absorption occurred regularly from the cervix in all cases in from nine to twenty-five minutes, while from the vagina it occurred irregularly and tardily in from twenty to 120 minutes.

We turn now to a consideration of absorption from the urinary bladder, serous cavities, spinal canal, eye, ear, breast, and skin, organs and tissues which, with the exception of the serous sacs, are not ordinarily considered to possess pronounced absorptive properties. Many have studied this phase of the subject using dyes, alkaloids, salts, inanimate

particles, and similar substances. Relatively few experiments have dealt with the absorption of proteins, and in these the usual immunologic or chemical methods have been employed.

I should like to present our findings with the direct technique in a series of studies on this subject which are as yet unpublished. For the sake of brevity it will be necessary to omit historical references and experimental details. In some instances only the conclusions will be presented. The technique employed may be briefly summarized as follows:

Passive local cutaneous sensitization to cottonseed was accomplished with a sensitizing serum obtained from an asthmatic subject with marked clinical and cutaneous sensitivity to this allergen. This serum presented a reaginic titer of 1:2048 by the dilution method of Coca and Grove. For sensitization, 0.05 ml. of undiluted serum or of serum diluted 1:4 or 1:10 was injected intracutaneously. In human beings the site of sensitization was the arm or forearm; in monkeys, the thigh was generally employed. For the twelve hours preceding sensitization and until testing was completed, the subjects were placed on a diet eliminating those foods (peas, beans, nuts, and mustard) for which reagins in high titer were present in the serum.

Testing for the absorption of cottonseed allergen was usually performed about forty-eight hours after sensitization of the cutaneous site. In human beings no breakfast was allowed on the morning of testing. In monkeys only water was allowed for the preceding twenty-four hours. When anesthesia was needed in monkeys, 1 or 2 gr. of nembutal were given intravenously. For testing, variable amounts of a cottonseed "milk," consisting of 10 Gm. of ground dehulled cottonseeds in 30 ml. of water, were used. When sterility was necessary, sterile cottonseed extract containing 0.57 mg. of nitrogen per milliliter was employed.

Absorption of cottonseed allergen from the urinary bladder was studied in monkeys and in human beings by Baretz and Harten.

*Catheterization being difficult in monkeys, it was necessary to resort to laparotomy and bladder puncture in the two animals studied. Test doses of 1 and 2 ml. of sterile cottonseed extract were introduced into the bladders. The sensitized cutaneous sites began to react in twenty and seven minutes, respectively. In the five human subjects studied, 2 ml. of cottonseed extract were introduced through a catheter into each bladder. The absorption times varied from ten to seventeen minutes, with an average of 13.8 minutes. In comparison to the rate of absorption of cottonseed from other mucous membranes, absorption from the bladder is relatively slow.

Absorption of cottonseed allergen from the serous-lined cavities of the body was studied by Harten, Gray, Livingston, and Friedman.

Three monkeys, averaging about 5 pounds in weight, were tested for the absorption of cottonseed from the peritoneal sac. In two animals, laparotomy was

*Small type represents summaries of lantern slides.

performed and 1 ml. of cottonseed extract was introduced into the abdomen. The absorption time was three minutes in each. In the third monkey, 0.1 ml. of extract was injected by needle puncture into the peritoneal sac. The absorption time was five minutes in this animal.

The absorption of cottonseed allergen from the pleural sac was studied in four monkeys. Sterile cottonseed extract (0.5 ml.) was introduced into the pleural space of each animal. In two monkeys this was performed by needle puncture without creating a pneumothorax. The absorption times were 4 and 4.5 minutes in these animals. In one monkey, a closed pneumothorax was created by the injection of 60 c.c. of air into the pleural space. The absorption rate in this animal was 5.5 minutes. In the fourth animal, in which an open pneumothorax was created by rib resection, the absorption time was seven minutes.

In three monkeys, absorption of cottonseed allergen from the pericardial sac was investigated. Following partial resections of the fourth, fifth, and sixth ribs, from 0.2 to 0.5 ml. of cottonseed extract was introduced by needle puncture into the unopened pericardial sacs. The absorption times were 6.5, 8, and 8 minutes, respectively, in these animals.

Although our animal series were small and the technique was not constant in the different experiments, the results obtained tended, in several respects, to confirm the findings of others who studied the absorption of noncolloidal substances from the serous sacs. Absorption of cottonseed allergen occurred rapidly, and at about the same rate, from the pleural and peritoneal cavities. Absorption from the pericardial sac was not quite so rapid. The presence of a pneumothorax tended to slow up absorption from the pleural sac to a slight degree.

There is little information available on the question of absorption of proteins from the mammillary ducts. This subject was studied in human beings by Livingston and Harten.

For testing, a 28-gauge needle with a blunted end was gently introduced into one of the duct openings at the nipple, and a small amount of cottonseed extract (0.2 to 0.3 ml.) was injected without pressure. No bleeding or discomfort followed this procedure. In five normal breasts, the absorption times varied from four to ten minutes, with an average of 7.2 minutes. In seven abnormal breasts (carcinoma, chronic cystic disease, adenosis, etc.), the absorption times varied from seven to fifteen minutes, with an average of 10.5 minutes. In a normal lactating breast, the absorption time was twelve minutes.

Albert and Harten studied the absorption of cottonseed allergen from the spinal canal in three monkeys.

Following lumbar puncture and the removal of from 0.3 to 1 ml. of clear spinal fluid, from 0.2 to 0.45 ml. of cottonseed extract was introduced. The needle was then plugged and left in situ until the cutaneous reaction appeared. This occurred in three, nine, and fourteen minutes, respectively.

Using the direct technique, Chait studied the absorption of peanut allergen from the eye and ear. For the conjunctival study, he used as a subject a 6-year-old girl who, at the age of 2 years, had had an extirpation of her lacrimal sacs for dacryocystitis. The eye appeared nor-

mal except for the tearing. For testing, a tiny pledget of cotton, dipped in peanut extract containing 0.5 mg. of nitrogen per milliliter, was inserted into the lower conjunctival sac. In twenty-one minutes, the previously sensitized cutaneous site reacted severely.

For the ear studies Chait used three nonatopic adults with normal membrana tympani. For testing, 1 ml. of peanut extract was instilled into the ear canal and kept there for forty-five minutes. In none of the three cases was absorption demonstrated.

In several studies, A. Walzer and Sack used the direct technique for the immediate detection in the circulation of proteins which had been rubbed into the skin. In one particularly interesting investigation they compared the absorption rates of cottonseed suspensions in petrolatum and in lanolin. For testing, 2 Gm. of dehulled cottonseed were triturated and incorporated in an equal amount of base. Most investigators had reported that, in the inunction of drugs in animals, lanolin as a base produced more rapid absorption than petrolatum. This proved to be true with the inunction of cottonseed in monkeys but not in human beings.

In comparative studies on three monkeys, the average absorption times for the lanolin and petrolatum suspensions of cottonseed were 7.2 and 12 minutes, respectively. In all animals cottonseed in a lanolin base was absorbed more rapidly than cottonseed incorporated in petrolatum. In comparative tests on twelve human beings, the absorption times were 17.2 and 17.6 minutes for the petrolatum and lanolin preparations, respectively. In six instances, the cottonseed in the petrolatum suspension was absorbed more rapidly. In five cases the lanolin preparation induced an earlier reaction. In one subject the absorption times were the same with both preparations.

Throughout all of these experiments we have been fully aware that only traces of allergens were being absorbed into the circulation. Although no method of measuring these small amounts of protein is available, recent investigations by Brunner and Baron have given us some information as to the quantities involved. They determined the smallest amount of cottonseed extract in the circulation needed to excite a sensitized cutaneous site.

Twenty-eight nonatopic subjects were sensitized with undiluted cottonseed serum. After two days these subjects were tested intravenously with varying amounts of cottonseed extract. It was found that with an intravenous injection of cottonseed extract containing 0.0001 mg. of nitrogen, positive reactions were induced at the sensitized cutaneous sites in five of the eleven subjects tested. This appeared to be the borderline dose, since with larger amounts of extract, containing from 0.00015 to 0.001 mg. nitrogen, positive reactions occurred in seven of eight subjects tested. With smaller amounts of extract, ranging from 0.000075 to 0.000025 mg. nitrogen, only one of the nine subjects tested developed a positive reaction. It is therefore reasonable to assume that, in the various experiments performed on human beings

with this particular serum, a positive reaction at the sensitized site indicated the passage of an amount of cottonseed allergen into the circulation equivalent to at least 0.0001 mg. of nitrogen.

In order to determine the amount of cottonseed which 0.0001 mg. of nitrogen represents, an extract was carefully prepared and titrated. It was found that one dehulled cottonseed of average size yielded 0.56 mg. of nitrogen and that 0.0001 mg. of nitrogen represents 1/5600 of one cottonseed. In peanut extract, 0.0001 mg. of nitrogen represents 1/44,000 of one peanut kernel.

Stevens, in a personal communication, stated that "in comparison with other native proteins, the cottonseed antigen is of relatively low molecular weight." The relatively small size of this molecule may be one of the factors responsible for its rapid absorption.

Since both Feinberg and A. Walzer found that a rapid absorption time usually marks the beginning of a sharply rising, high antigen tide, it is likely that, in the case of cottonseed, the total amount of allergen which enters the blood stream may be several or many times greater than 0.0001 mg. of nitrogen, which is the minimal amount needed to light up the previously sensitized site.

Ever since Bernard did his pioneer experiments showing that the liver may remove proteins from the blood, it has been maintained that this organ forms a barrier which prevents any undigested protein from reaching the general circulation by way of the portal vein. Failure or impairment of this alleged protective function formed the basis for many theories as to the cause for the development of food allergy. This question seemed sufficiently important to warrant further investigation, and experiments testing the filtering power of the liver for cottonseed protein were performed in monkeys.

A series of monkeys were given intravenous (femoral) doses of varying amounts of cottonseed antigen to determine the amount needed to light up cutaneous sites which had been sensitized with cottonseed serum. All determinations, in this series of experiments, were made while the animals were under an anesthetic (nembutal). It was found that the borderline dose of cottonseed extract was in the neighborhood of 0.0001 mg. of nitrogen. This amount of cottonseed when given to six animals gave four positive and two doubtful reactions, the average reaction time for the positives being 5.8 minutes. Of four animals receiving 0.000075 mg. of nitrogen, which was less than the borderline dose, two gave positive reactions and two negative, the reaction times for the positives being eleven minutes. In eleven animals receiving 0.00015 or 0.0002 mg. of nitrogen, which was slightly more than the borderline dose, ten gave positive reactions and one a doubtful reaction. The average reaction time for this group was 4.9 minutes. When, after an interval of from twelve to twenty-six days, eleven of these animals were given doses of 0.00015 or 0.0002 mg. of nitrogen into the portal vein, ten gave positive reactions and one a negative. The average reaction time for this group was 5.8 minutes. From these results, it was apparent that the liver did not remove a large part of the cottonseed

from the blood in the portal vein, since the percentage of positive reactions as well as the reaction times were not strikingly affected by the portal administration of the allergen.

From these experiments, it is apparent that, while the liver may remove some antigen from the blood in the portal vein, it by no means forms a complete or effective barrier to the passage of significant amounts of allergens into the circulation. As the experiments of Alexander, Drinker, Yoffey, and many others indicate that proteins readily reach the blood stream by way of the lymphatic system, little evidence remains to support the theory that the liver plays a vital role in the prevention of sensitization and of allergic reactions by filtering allergen out of the blood in the portal vein.

Absorption of allergens from the respiratory tract is as important in allergy as is absorption from the alimentary tract. The experiments of Ratner, Alexander, and others dealing with respiratory anaphylaxis in the guinea pig and the experiments of Simon and Rackemann on respiratory sensitization in human beings are well known. The direct method of demonstrating the absorption of allergens from the respiratory tract was first employed by Cohen, Ecker, Breitbart, and Rudolph in Connection with pollens and later by Sulzberger and Vaughan with powdered silk. In these studies the antigen was sprayed, blown, or insufflated into the nose, and the site of absorption as well as the amount absorbed could not be controlled. These technical difficulties were surmounted to some extent in a modification of the direct method which has recently been developed by Chait. In this technique, testing for absorption is done by the application to the anterior portion of the nasal septum of a measured amount of antigen solution, which is contained in a piece of a compressed cotton pad of standard size and consistency.

The anterior inferior portion of the septum was chosen as the site for application of the antigen because it presents many advantages. It is visible to the investigator and is easily accessible. It is a flat surface to which the flat antigen-soaked tab readily clings. A square centimeter of a compressed cotton pad (such as cottonoid*), when moistened with exactly 0.25 ml. of peanut extract, will adhere to the septum with virtually no leakage of the fluid. Moreover, preliminary studies revealed that absorption from the septum occurs with the same degree of regularity and almost as rapidly as absorption from the mucous membranes under the turbinates.

In sixteen subjects, the application under the inferior turbinate of a cotton applicator (the flat cotton pad could not be used on this surface) moistened with cottonseed extract (0.5 mg. of nitrogen per milliliter) resulted in an average absorption time of 12.1 minutes. In an equal number of cases, the average absorption time following a similar application under the middle turbinate was 13.9 minutes.

*Johnson & Johnson.

The rate of absorption from the anterior portion of the septum in twenty-eight subjects, on whom the standard cotton pad technique was employed, averaged 14.2 minutes.

The development of a standard technique by Chait made it possible to investigate an important controversial question in allergy; namely, whether absorption is altered in allergic individuals.

In order to do comparative studies on atopics and nonatopics, it was necessary to obtain subjects in each group who were equally receptive to passive local sensitization with the particular serum which was to be employed. Titrations of the cutaneous receptivity of these subjects were made by Bowman. A control group was carefully selected. The atopic group consisted of frank cases of hay fever with positive skin reactions to pollens. The absorption tests were done at seasons when the nasal mucous membranes were normal in appearance and when the patients were completely free of allergic or infectious nasal symptoms.

Seventy-six atopic and sixty-nine nonatopic subjects were sensitized with peanut serum in dilutions of 1:4, 1:16, 1:64, 1:256, 1:1024, and 1:2048. Forty-eight hours later the sensitized sites were tested intracutaneously with peanut extract containing 0.01 mg. of nitrogen per milliliter. Acceptance of transfer at a sensitized site was indicated by the development of a one plus or stronger reaction as a result of the test. Among the normal subjects, acceptance with the higher dilutions, i.e., 1:256 or more, occurred in 94.2 per cent of the cases. Among the atopic subjects, only 47.4 per cent fell into this category. Since acceptance in a high dilution (at least 1:256) is necessary for a proper study of the absorption phenomenon, only one-half as many atopics as normals were available for the absorption experiments.

Nasal absorption tests were performed on forty nonatopic and twenty atopic subjects whose adequate acceptance of cutaneous sensitization with the peanut serum had been established. Employing his standard technique, Chait found that in each group one subject failed to show an absorption reaction. Of the fifty-eight positive reactors in the two groups, all but seven showed absorption times ranging from seven to twenty minutes. These were therefore considered the limits of normal absorption times. Absorption times longer than normal occurred in two nonatopic and five atopic subjects. Hence, 92.5 per cent of the nonatopic subjects and 70 per cent of the atopic subjects had normal absorption times. Among these the average absorption time was thirteen minutes for the nonatopic individuals and fifteen minutes for the atopic individuals. The most rapid absorption time among all the cases (seven minutes) occurred in an atopic subject.

Summarizing this experiment, it would appear that about 70 per cent of the hay fever subjects, who accepted cutaneous sensitization adequately, showed nasal absorption rates for the peanut antigen which fell within normal limits, whereas about 30 per cent showed either negative or delayed absorption rates. This failure of the latter group to absorb peanut antigen normally cannot be taken as an absolute measure of their absorptive powers, since some of these atopic subjects, at another time, demonstrated normal nasal absorption rates with cottonseed allergen. On the basis of this information, the assumption that absorption is diminished in all or even in a majority of atopic individuals is without foundation.

Some allergists are still laboring under the false impression that the absorption of protein in an undigested state occurs only occasionally or following the ingestion of a few potent allergens, such as fish, eggs, and nuts. On the contrary, it seems likely that traces of most ingestants and inhalants are almost constantly entering the blood stream in an unaltered state. *The absorption of almost any food or inhalant can be demonstrated if a serum of sufficiently high reaginic titer can be obtained for the purpose.* This was brought out in an analysis of the charts of several hundred allergy cases which were tested by the indirect method by Bowman. It was a common occurrence for the sensitized cutaneous sites on substitutes to be excited accidentally by the ingestion of various foods, some of which are not ordinarily considered to be potent allergens.

Among the allergens which do not commonly excite the sensitized sites, absorption reactions occurred with broccoli, banana, pumpkin seed, milk, spinach, turkey, and chicken. All of these had been taken by the substitute during the course of an ordinary meal, with no expectation that their ingestion would excite the sensitized sites. The allergens which most commonly offended in this respect were egg, nut (many varieties), fish (many varieties), pea, and mustard. There no doubt are other allergens which have caused unexpected reactions in the course of passive transfer studies by other workers.

In closing, then, I wish to re-emphasize the fact that the absorption of unaltered protein into the circulation is a normal, physiologic phenomenon occurring in nonatopic as well as atopic individuals, at all ages, and with many allergens. The facts concerning this absorption must be taken into consideration in the evaluation of theories of food sensitization. Keeping them in mind should also be of some assistance in the practical management of allergic illnesses.

COMPARATIVE IMMUNOLOGIC STUDIES WITH SALIVARY AND EPITHELIAL EXTRACTS OF THE DOG, CAT, AND RABBIT

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IT IS a common observation that individuals hypersensitive to animals frequently exhibit hives, urticaria, localized erythema, itching, and edema on skin areas previously licked or mouthed by pets. That such reactions amount to actual scratch or patch tests of the saliva of these animals seemed probable. Cooke[1] mentions the fact that dog saliva may act as an excitant, but a review of the literature failed to reveal any experimental studies on the antigenic properties of saliva even though much work has been done on its electrolyte,[2] enzyme,[3] and blood group specific antigen[4] content. Furuhata[5] showed that saliva in such small amounts as may be present on discarded cigar butts found at the scene of a crime could be used to determine the blood type. Group antigens were found to be present in the salivary secretion itself and were not due to the presence of epithelial cells or blood.[4] Therefore, experiments were devised to study the antigenic interrelationship of epithelial and salivary extracts. Patients showing positive intracutaneous tests to dog, cat, or rabbit epithelial extracts were also tested with the respective salivas. Serum from patients sensitive to dog, cat, or rabbit was obtained for passive transfer studies. After virgin guinea pigs were sensitized with the epithelial and salivary extracts, their uterine horns were tested in the Dale bath with the two antigenic extracts of the species used for sensitization.

METHODS

Salivary Extracts.—Animals were not allowed access to food during the night and were used the next morning. This precaution prevented the regurgitation of food which otherwise occurred during the collection of saliva. All animals were anesthetized by the intraperitoneal injection of sodium pentobarbital, approximately 30 mg. per kilogram of body weight. The animal's mouth was then washed with distilled water to remove any loose food debris. One cubic centimeter of a solution containing approximately $\frac{1}{40}$ gr. of pilocarpine was injected subcu-

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taneously. If the flow of saliva was slow, a second injection of pilocarpine was sometimes given one-half to one hour after the first. The anesthetized animals were kept warm with covers and were so placed that the head was lower than the rest of the body. This position was assumed in order to prevent the aspiration of saliva and death from asphyxia or subsequent pneumonia. A wooden tongue depressor was placed between the teeth of the animal, and the saliva flowed down this depressor into a suitable receptacle.

All samples of saliva were acidified with hydrochloric acid to destroy the ptyalin, and this also precipitated out a great part of the mucin. The saliva was filtered, neutralized with sodium hydroxide, and then dialyzed against buffered saline for twenty-four hours. The saliva was concentrated by allowing it to evaporate at room temperature in cellophane tubing in the vicinity of an electric fan. The salivary extract was then passed through a Seitz filter and standardized according to its content of protein nitrogen.

Epithelial Extracts.—Epithelial extracts were obtained from scrapings of the hair and loose epithelia of the skins of the various animals. After a fat extraction, the epithelia were extracted with buffered saline and the extract was dialyzed, concentrated, Seitz filtered, and standardized as described above.

SKIN TESTING ON SENSITIVE INDIVIDUALS

Individuals clinically sensitive to dog, cat, or/and rabbit were tested by the intracutaneous injection of 0.025 c.c. of the salivary and epithelial extracts in a strength of 100 or 1,000 units per cubic centimeter. Closely parallel skin reactions were obtained to the salivary and epithelial extracts of the corresponding species, and no individuals were observed who were sensitive to the one who did not react to the other. Patients sensitive to only one or two of the above-mentioned species were, however, tested with all three. In a few cases, however, more active skin reactions were obtained with the one extract than with the corresponding extract.

NEUTRALIZATION OF IMMUNE SERA BY SALIVARY AND EPITHELIAL EXTRACTS

Sera from individuals sensitive to dog, cat, and/or rabbit epithelia were passed through a Seitz filter to insure their sterility. Observing sterile precautions, equal volumes of undiluted sera and extract were mixed, placed in an incubator at 37° C. for one hour, and then stored in the icebox. Depending somewhat upon the amount of skin sensitizing antibody present in each individual serum, varying concentrations (25, 50, 100, 200, 400, 600 units per cubic centimeter) of antigenic extract

were used. It was always necessary for the satisfactory titration of the neutralization point to have a series of mixtures in which insufficient antigen had been added to the serum so that a test of this passively sensitized site would give a positive result. In the last of the series of mixtures, sufficient antigen had been added to the serum to neutralize all of the antibody so that a test of this passively sensitized site would result in a negative reaction. Controls of equal volumes of serum and saline were run in all cases. Each recipient of the serum-antigen mixture was previously tested with 2,000 units per cubic centimeter of antigen extract, with negative results, to insure the selection of a nonsensitive individual.

Each serum-antigen mixture was placed in duplicate intracutaneous sites on the back of normal nonsensitive individuals. After twenty-four or forty-eight hours, one site was tested with 0.025 c.c. of 2,000 units per cubic centimeter salivary extract while the duplicate site was tested with the corresponding epithelial extract of the same strength. In this way each mixture site, whether salivary or epithelial antigen-serum mixture, was tested with both salivary and epithelial extract in order to study any quantitative difference in the two extracts.

Approximately twenty-four sites were necessary when only one species was tested, but since most sera were from individuals who showed multiple sensitivity, usually extracts of two or more species were tested, making forty-eight or more sites.

Table I shows that the same protein nitrogen unit strength of dog saliva is just as capable as dog epithelium extract in neutralizing the skin-sensitizing antibodies present in human allergic serum. The neutralization point was almost identical in this particular experiment for both dog saliva and dog epithelial extracts, and the same neutralization point was observed in another recipient with this same mixture. It was necessary for about 200 units of each extract to be mixed with the serum before neutralization was accomplished, and approximately this same neutralization point was obtained whether the antigen-serum mixture sites were tested with dog saliva or dog epithelial extracts. Similar tests were obtained with cat saliva and cat epithelium shown in the lower part of Table I. Not all serum mixtures were neutralized with the low concentration of antigen shown in this table. Sometimes antigen strengths of 600, 800, and 1,000 units were employed, depending upon the antibody strength of the serum used. Not all sera tested resulted in identical neutralization tests, but most of the neutralization points were within 200 units of each other, which would seem to be within the limit of error of the method of testing. As was to be expected, all control sites of serum and saline gave marked reactions on test.

TABLE I

MIXTURE NUMBER	NEUTRALIZATION		TEST ON RECIPIENT A		TEST ON RECIPIENT B	
	SERUM MIXED WITH	UNITS PER C.C.	REACTION ON TEST WITH 1,000 UNITS PER C.C.		REACTION ON TEST WITH 1,000 UNITS PER C.C.	
			DOG EP.	DOG SAL.	DOG EP.	DOG SAL.
1	Dog ep.	25	Marked	Marked	Moderate +	Marked
2	Dog ep.	50	Marked	Marked	Moderate	Moderate
3	Dog ep.	100	Moderate	Moderate to marked	Slight	Slight
4	Dog ep.	150	Slight	Slight	Slight	Negative
5	Dog ep.	200	Negative to slight	Negative	Negative	Negative
6	Saline		Marked	Marked	Moderate +	Marked
7	Dog sal.	25	Marked	Marked	Marked	Moderate +
8	Dog sal.	50	Marked	Marked	Moderate	Moderate
9	Dog sal.	100	Moderate	Slight	Moderate	Slight
10	Dog sal.	150	Slight	Negative	Slight	Negative
11	Dog sal.	200	Negative to slight	Negative	Slight	Negative
12	Saline		Marked	Marked	Marked	Marked
MIXTURE NUMBER	SERUM MIXED WITH	UNITS PER C.C.	REACTION ON TEST WITH 1,000 UNITS PER C.C.		REACTION ON TEST WITH 1,000 UNITS PER C.C.	
			CAT EP.	CAT SAL.	CAT EP.	CAT SAL.
1	Cat ep.	25	Moderate	Moderate	Moderate +	Moderate
2	Cat ep.	50	Slight	Slight	Moderate	Slight
3	Cat ep.	100	Slight	Slight	Slight	Negative
4	Cat ep.	150	Negative	Negative	Negative	Negative
5	Cat ep.	200	Negative	Negative	Negative	Negative
6	Saline		Marked	Marked	Marked	Marked
7	Cat sal.	25	Moderate	Moderate	Moderate +	Moderate
8	Cat sal.	50	Slight	Slight	Slight	Slight
9	Cat sal.	100	Negative	Negative	Negative	Negative
10	Cat sal.	150	Negative	Negative	Negative	Negative
11	Saline		Marked	Marked	Marked	Marked

A retest was made on the tested sites shown in Table I. Those sites previously tested with epithelium were retested with saliva and vice versa. Mixture sites, previously negative to one antigen failed also to respond to the corresponding antigenic extract of the same species. This tended to show that there was no extra antigenic fraction in one of the extracts (salivary or epithelial) which could elicit a reaction from human allergic serum already neutralized by the other extract.

COMPARATIVE STUDIES WITH SALIVARY AND EPITHELIAL EXTRACTS ON THE SENSITIZED GUINEA PIG

Virgin guinea pigs of approximately 300 Gm. weight were sensitized by the intraperitoneal injection of approximately 60,000 units of salivary or epithelial extracts of the dog, cat, or rabbit.

The guinea pigs were used approximately three weeks after the sensitizing dose of antigenic extract was administered. They were killed by a blow at the base of the skull, and the uterus was removed with care

and placed in Tyrode's solution. The uterine horns were then tested in a Dale type oxygenated bath of Tyrode's solution with constant temperature.

One uterine horn was always tested not with the specific antigen used for its sensitization but with the related antigen of the same species. Fig. 1 shows a uterine horn sensitized to rabbit saliva and first tested with rabbit epithelium. After contraction, the chamber was washed with fresh Tyrode's solution, and, after subsequent relaxation had occurred, the process was repeated until rabbit epithelium no longer elicited a response. Then the specific antigen, in this case rabbit saliva, was added to the muscle bath. Epithelial and salivary extracts of other species to which the guinea pig had not been sensitized were tested first, and no nonspecific reactions to these antigens were encountered. Fig. 1 shows that a guinea pig uterine horn sensitized with rabbit saliva gave marked contractions upon the addition of rabbit epithelium to the muscle bath. Nevertheless, after exhaustion of this response to rabbit epithelium, a marked reaction was obtained upon the addition of rabbit saliva to the muscle bath.

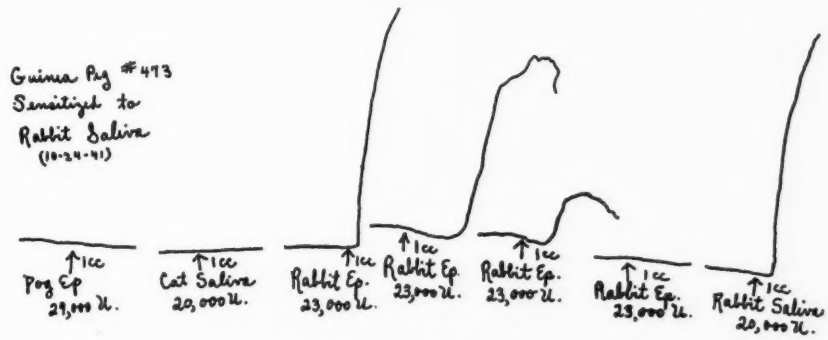

Fig. 1.

Caution should be exercised in drawing conclusions solely from the experiments cited above, that there is an extra antigenic factor present in saliva which is not neutralized by epithelium. For example. Fig. 2 is the kymographic tracing of two horns from the same animal which was sensitized to cat saliva. It will be noted that no response whatever was obtained upon the addition of cat epithelium to the muscle bath of horn No. 1, whereas horn No. 2 gave a marked reaction to cat epithelium. This reaction completely blocked any further reaction of this horn, even to cat saliva to which the animal was originally sensitized, and a marked reaction to histamine showed that the muscle was still capable of reacting had any antibody been present.

There were eighteen pigs in all whose duplicate horns were both tested first not with the specific antigen used for its sensitization but with

TABLE II

1	2		3		4		5	
GUINEA PIG SENSITIZED WITH	REACTION TO SALIVA (FIRST TEST)		REACTION TO EPITHELIUM (AFTER EXHAUSTION TO SAL.)		REACTION TO EPITHELIUM (FIRST TEST)		REACTION TO SALIVA (AFTER EXHAUSTION TO EP.)	
Dog epithelium	442	++++	442	++	440	++++	440	+
	452	++++	452	++++	452	++++	452	+
	440	++++	440	-	281	++++	281	-
	441	++++	441	-	441	++++	441	-
	453	++++	453	-	453	++++	453	-
	499	++++	499	-				
	300	+	300	-				
	299	++++	299	-				
	245	++++	245	-				
	245	++++	245	+				
	230	+	230	+				
	230	++++	230	++++				
	244	++++	244	-				
	244	++++	244	-				
Cat epithelium	262	+	262	++++				
	262	-	262	+				
	261	++	261	++				
	261	++	261	-				
	250	++++	250	-				
	250	++	250	+				
	490	++	490	+++				
Rabbit epithelium	276	++++	276	+	276	++++	276	-
	277	++++	277	-	277	++++	277	-
	275	-	275	+++				
	275	++++	275	+				
	474	-	474	++++				
	474	-	474	+++				
Dog saliva	457	++++	457	-	457	++++	457	++++
	296	+++	296	-	458	++++	458	++++
					483	-	483	++++
					483	-	483	++++
					484	-	484	++++
					485	-	485	+
					485	-	485	++
					298	++++	298	-
					246	++++	246	-
					246	++++	246	++++
Cat saliva	432	++++	432	-	432	++++	432	++++
	433	++	433	-	433	+++	433	++++
	448	++++	448	-	434	-	434	++++
	272	++++	272	-	272	-	272	++++
	274	++++	274	-	274	-	274	++++
					430	++++	430	-
					449	+	449	-
					449	-	449	++++
					293	-	293	+++
					293	+++	293	-
					291	-	291	+
					273	-	273	+
					273	-	273	++++
Rabbit saliva					473	++++	473	++++
					278	-	278	++++
					278	-	278	++++
					279	-	279	++++
					279	-	279	++++
					280	-	280	++++
					225	-	225	+++
					225	-	225	++
					472	-	472	++++
					472	-	472	++++

the related antigen of the same species. There were only four animals whose duplicate horns reacted differently to the first test antigen, as shown in Fig. 2.

Occasional variations of this type must be considered as within the limit of error in this method of biologic assay. However, cross reactions with saliva or epithelium from different species were not obtained, showing the definite specificity which can be obtained by this procedure.

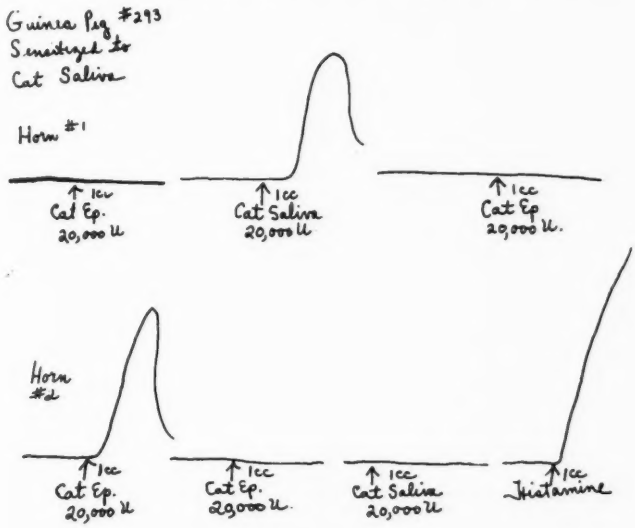

Fig. 2.

The seventy-four individual horns whose reactions were exhausted to one antigen before testing with the related one are tabulated in Table II. Column 2 shows that the great majority of guinea pigs sensitized to epithelium gave marked reactions to saliva as the first test. Only four negative saliva reactions were recorded in a series of twenty-seven uterine horns. After the reactions to saliva were exhausted, these horns were tested with epithelium. Results are shown in Column 3. Approximately one-half of the horns responded to epithelium; the other half was negative to epithelium due to the complete exhaustion of the antibodies in the epithelium-sensitized horn by the related salivary extract.

Column 4, however, shows that the majority of the guinea pigs sensitized to saliva did not react to the epithelium extract. There were only eleven animals which responded to epithelium, whereas twenty-two gave negative results. After the reactions to epithelium had been exhausted, these horns were tested with saliva. Results are shown in Column 5. It must be recalled that twenty-two of these saliva-sensitized animals did not originally respond to the related epithelial extract so that if they were sensitized at all they would naturally respond to the saliva when tested with it. Of the eleven animals which gave positive

reactions to the epithelial extract, five failed to react on later test with saliva. This demonstrated again that approximately one-half of the horns were negative to saliva due to the complete exhaustion of the antibodies in the saliva-sensitized horn by the related epithelial extract.

Animals sensitized to epithelium and tested first with epithelium never failed to give a positive result (seven horns, Column 4). Upon exhaustion of these epithelial responses, a faintly positive saliva test was elicited in two animals, the other five being completely negative (seven horns, Column 5). Likewise animals sensitized to saliva and tested first with saliva never failed to give a positive result (seven horns, Column 2). Upon exhaustion of these salivary responses, no positive epithelial test could be elicited (seven horns, Column 3).

Table II shows that guinea pig horns sensitized to epithelium gave marked contractions to saliva in twenty-three cases out of twenty-seven as the first test. However, guinea pigs sensitized to saliva gave marked reactions to epithelium in only eleven cases out of thirty-three. In other words, while practically all guinea pig horns sensitized to epithelium reacted to saliva as the first test, less than one-half, or about one-third, of the saliva-sensitized horns responded on test to epithelium. This would seem to suggest the presence of some extra antigenic factor or factors present in the salivary extracts.

For example, let us suppose that the epithelial extract has the antigenic factor A present and that the salivary extract has the A factor plus a B, C, and/or D factor. Obviously, an animal sensitized with an epithelial extract containing only factor A will react to test with saliva which also contains fraction A. However, an animal sensitized with a salivary extract containing fraction A and B, C or D will contain antibodies to A and B, C or D, and the quantity of fraction A antibody will be much less than that produced by an animal sensitized to epithelium which contains only fraction A. This would explain the large number of negative responses to the epithelium in a saliva-sensitized animal and explain the possibility of some positive reactions due to sufficient antibody A, in some cases to elicit the response.

Witebsky and Werner[6] prepared immune sera by injecting rabbits with human saliva and serum, respectively. With complement fixation, these sera gave the strongest reaction with the homologous antigens and weak or no reactions in cross test, showing that saliva and serum could be differentiated. We are now attempting to repeat this type of experiment, using the salivary and epithelial extracts of the dog and cat to study the precipitin or blocking strength of one antigen to the related antisera.

DISCUSSION

In the household where animals are kept, certainly saliva must be deposited on furnishings previously licked or mouthed by these pets.

It is thus of clinical interest that there is a common antigenic factor present in the salivary and epithelial extracts of the dog, cat, and rabbit. The foregoing experiments tend to indicate that therapy with the epithelial extract should also satisfactorily cover the corresponding salivary antigen. Results from this standard type of allergic treatment confirm this view.

It should be noted that due to the manner and degree to which household animals are continually licking their fur to clean themselves, the hair, or fur, must usually be contaminated with saliva. Hence, it is the mixture of these two excitants rather than the epithelium alone which causes the clinical symptoms in the animal-sensitive patient. A small amount of salivary extract must be present in any ordinary epithelial extract. That this quantity could in any way account for the closely parallel reactions obtained with the corresponding salivary and epithelial extracts seems unlikely. There certainly would not be enough salivary extract present in the epithelial extract to produce in the epithelial-sensitized guinea pig enough antibodies to saliva to give such regularly positive cross test with the salivary extract.

SUMMARY

1. A common antigenic factor has been shown to be present in epithelial and salivary extracts of the dog, cat, or rabbit by the following experiments: (1) direct skin tests on patients hypersensitive to animals; (2) neutralization point determinations on mixtures of sensitive serum and epithelial or salivary extracts; (3) studies of the reactions of guinea pig uteri sensitized to epithelial and salivary extracts.

2. The presence of a possible extra antigenic fraction in the salivary extract is discussed.

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DISCUSSION

DR. ALBERT VANDER VEER.—I want to congratulate Dr. Spain, Dr. Gillson, and Miss Strauss for a very interesting and useful paper. If you have ever seen these pets around the house you have seen a cat licking herself half the time and if you show a dog a bone and if he does not get it, he drools all over the place. There must be a good deal of saliva mixed in with house dust which might bother sensitive people.

A good many years ago we had an extract of dog saliva and we tried skin testing with it. It gave very marked skin reactions, so much so that we were very careful in its use. After we had used up that supply we never got any more.

If what this paper shows is true, then it cuts our work in half in treating patients who are sensitive to dogs and cats. We will not have to fuss with two preparations. If we use epithelium extracts that will accomplish our purpose.

The authors did not go into the question of horse saliva and horse epithelium, but I have no doubt that that would probably hold true also. In the past I have run across a couple of cases of horse-sensitive people who said they were very much worse when they were riding a horse than when they were driving behind it. Their eyes and faces would itch and they would sneeze. In other words, they were getting the full benefit of the saliva at close range and I have no doubt that the epithelium will protect against the saliva as well.

DR. GILLSON.—For some time I have been very interested in the work that has been prepared and done by Miss Strauss, and in following the work she has had to do I can assure you that she has had plenty of opportunity for trouble. In each branch of the work there is a great deal of opportunity for error. Since each step is dependent on the preceding, each step must be accurately worked out to satisfaction before the progressive steps can be taken in order. If there is any inaccuracy at any one point, it then throws the rest of the work entirely out of the picture and that means a repetition.

I do not want to go into too much detail but I would like to point out some of the difficulties Miss Strauss has been up against. I will put it under three headings and say a little about each.

In the beginning one must secure the epithelium extracts. This is easy, but on the other hand, when one has to obtain the saliva, that is a different matter. In the Postgraduate Hospital, where this work was done, no dogs were allowed. That made it very hard. We had to get around it, so we smuggled a pup in. After considerable work, it was found that a small dog, under 4 kilograms, would give about three to three and one-half ounces of saliva. That meant that we had to tap the dog, as it were, on several occasions in order to get a sufficient quantity to permit us to work. About a quart of that material, when properly concentrated and finished, and ready for use, would yield about 180 c.c. and a large quantity of that, of course, would have to be used in the Dale bath at 1 c.c. at a time and also a considerable quantity would have to be used for sensitizing the animals. We had a little difficulty in concentrating this material, because we found that it was necessary to get a protein nitrogen standard of at least 20,000 units. Less than that amount did not work so well in the Dale bath. At the same time, if we used a weaker solution than this, it meant that we had to use a greater number of cubic centimeters to get, say, 60,000 units into a guinea pig for the necessary steps of sensitization.

The sensitization of the guinea pigs was quite a problem in itself. We could not understand why so many of these animals died. Perhaps that may come out in the discussion later.

So far as the Dale machine was concerned, this apparatus also gave opportunity for considerable error. This equipment had to be properly stabilized and fixed up with the necessary connections and the kymograph and everything required for its use, and great care had to be

taken not to jar or do anything that would unduly excite the muscle we might be using. There was also the chance of error in handling the guinea pig horns. At the time these horns were taken from the guinea pigs, they were immediately placed in Tyrode's solution and care was exercised to avoid any possible trauma in handling.

Again there was a great deal of chance for error in the neutralization setup. In this particular instance, we had to be careful in the amount and quantity of the antigens that we used. We had to be careful with the dilutions. Time will not permit further discussion.

STUDIES IN FOOD ALLERGY

II. Sensitization to Fresh Fruits: Clinical and Experimental Observations

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Philadelphia, Pa.

IT IS a well-recognized and generally accepted fact that allergy to fruits is a frequent cause of allergic disturbances, particularly of the cutaneous variety. Urticaria, angioneurotic edema, burning or itching sensations of the mouth, canker sores, and diarrhea are the usual types of disturbance caused by such sensitization, although it occasionally may be responsible for such manifestations as migraine, allergic coryza, or even asthma. Symptoms more often follow the eating of the raw fruit than when it is cooked, canned, or preserved. This explains the greater incidence of urticaria in the summer when more fresh fruits are ingested than at any other time of the year.

Almost any type of fruit may cause symptoms of allergy; however, statistical evidence as to the frequency is quite variable. This is undoubtedly due to the fact that most of this evidence is obtained from the results of skin tests rather than from clinical findings. Fruit allergens used for skin testing usually are prepared in a rather concentrated solution, yet negative skin reactions often are obtained in individuals who give definitely positive clinical histories and in whom the symptoms may be induced by ingestion of the suspected fruit. Attempts have been made to explain this discrepancy usually upon the basis of the symptoms being produced by some intermediate—or split—product, but as yet no acceptable evidence has been offered in substantiation of these claims. Other related facts awaiting suitable explanation are that the skin of the fruit (e.g., orange or lemon peel or peach skin) may provoke symptoms in some patients when the edible portion of the fruit does not; also that group allergies exist, for example, among all citrus fruits or to apples and pears or to peaches, cherries, apricots, and plums.

Our interest in this particular phase of food allergy began in 1936 while studying three patients who repeatedly displayed symptoms typical of food allergy following the ingestion of certain fresh fruits. Skin tests, however, even with the concentrated stock extracts of these fruits were consistently negative. We decided at that time to test the patients with the freshly extracted juices. To our surprise markedly

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positive reactions were obtained. This formed the basis of the further studies which are included in the present initial report.

Before detailing the results of these studies, it might be well first to review briefly the clinical histories of these three patients as well as that of a fourth more recent addition belonging to the same group.

CASE REPORTS

CASE 1.—E. L., female, aged 24 years, was first seen on July 9, 1938, because of asthma beginning in childhood and recurring each week since. Each spell lasted from five to fifteen minutes and subsided spontaneously. In 1926 she developed typical fall hay fever and in 1933 mild spring hay fever, lasting three weeks during the month of May. She was subject to paroxysms of sneezing while riding a horse. The ingestion of fresh fruits (watermelon, honeydew melon, cantaloupe, pears, peaches, pineapple, and grapes) produced swelling of the mouth and throat. This occurred throughout the year but was more noticeable during the hay fever seasons. This peculiar reaction to fresh fruits (except plums) was first noted in 1927. Canned or stewed fruits failed to produce the reaction.

CASE 2.—V. B., male, aged 45 years, was first seen April 20, 1936, with fall hay fever present since 1922 and complicated by pollen asthma in 1929. The ingestion of pears, peaches, cantaloupe, bananas, oranges, and grapes caused an itching of the soft palate that was much more noticeable during the pollen season than at any other time. There was no aggravation of this hay fever syndrome following the ingestion of these foods. Canned and stewed fruits failed to produce this reaction.

CASE 3.—J. R., male, aged 40 years, was first seen on April 9, 1936, because of hay fever that began in 1929 and lasted from late April to late June and from mid-August to late September. Mild pollen asthma accompanied the fall hay fever. Tightness of the neck and itching of the throat followed the ingestion of oranges, watermelon, peaches, and cantaloupes. This was particularly annoying during the hay fever seasons, being mild and negligible at other times of the year.

CASE 4.—D. H., male, aged 23 years, medical student, was first seen on Feb. 6, 1941, because of severe seasonal hay fever and asthma that started in childhood and recurred from early April to late June. He experienced swelling of the mucous membrane of the mouth following ingestion of fresh green peas, apples, and peaches. Cooked or canned fruits failed to produce this swelling.

PROCEDURES AND RESULTS

Direct skin tests with the freshly extracted juice of most of the clinically reacting fruits were performed in the above patients. The juice used in the tests was obtained by first removing the skin of the fruit, then squeezing the whole fruit or its slices through ordinary gauze over a beaker and centrifuging the filtered material. The supernatant fluid then was removed and used in the skin tests as the fresh juice. Strict asepsis was not possible, but the operation was carried out as carefully as possible to avoid contamination. That little or no bacterial contamination occurred is indicated by the fact that not a single instance of infection was encountered in spite of the large number of

tests done with the fresh juice obtained in this way. Whenever possible and unless the conditions of the experiment demanded otherwise, the material always was stored in the refrigerator to prevent bacterial growth.

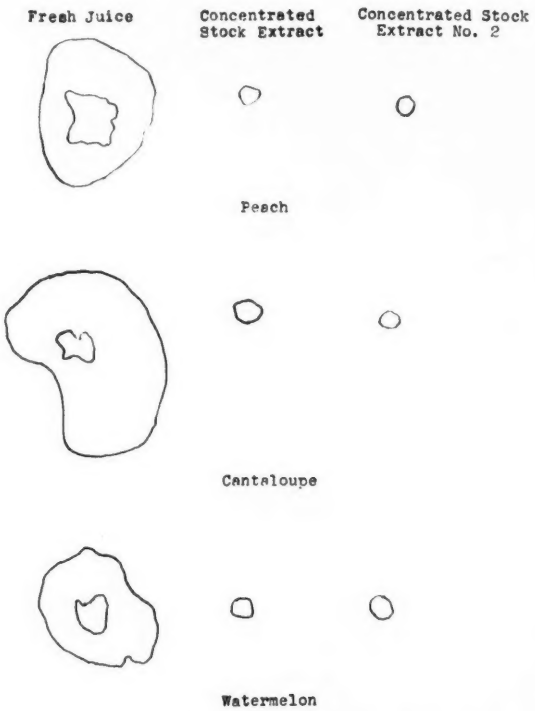

Fig. 1.—Direct skin tests on E. L.

As already stated, we were considerably surprised when definitely positive skin tests were obtained in the first three patients with the freshly extracted fruit juices to which they were clinically sensitive (including peach, apple, pear, pineapple, plum, cantaloupe, watermelon, and grape). On the other hand, similar tests with the concentrated stock extracts, both in the liquid and powdered states, were negative. By contrast, positive reactions to both the stock extract and freshly prepared juice of tomato were obtained in the patient in Case 2. The tomato juice was tested because it induced similar clinical symptoms after ingestion. The patient in Case 4, seen first in 1941, was tested only with peach and apple extracts. He likewise showed positive reactions to the fresh juices and negative reactions to the stock extracts.

The type of reaction occurring in the patient in Case 1, who was tested with the freshly extracted juice, is illustrated in Fig. 1 and may be taken as representative of the entire group. In order to be certain that failure to get reactions with the stock extracts was not attribu-

table to their impotency, the patients also were tested with the concentrated stock extracts obtained from two other allergy laboratories (L. C. and A. B.). The latter reacted in the same manner as our own extracts (Figs. 1 and 3). Control skin tests with the fresh juices likewise were performed upon a group of normal nonallergic persons and a group of allergy patients with negative clinical histories so as to determine the specificity of the reactions. Negative reactions were obtained in all instances, indicating that the material used was nonirritating and that the positive reactions were specific.

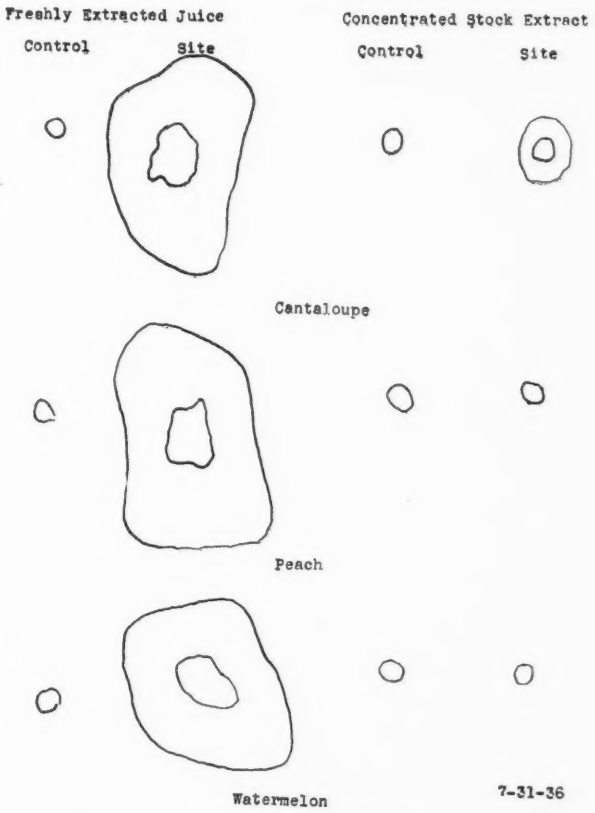

Fig. 2.—Passive transfers with E. L. serum.

Blood was withdrawn from all of these patients and the serum used for passive transfer by the usual Prausnitz-Küstner technique. Positive reactions similar to those of the direct skin tests were obtained in all instances in the sensitized sites with negative reactions in the nonsensitized or control sites, as illustrated in Fig. 2. It was evident, therefore, that sufficient reagins were present in the serum of these patients to induce passive sensitization.

These findings, obtained originally in 1936, were considered extremely significant at that time, but it was deemed inadvisable to report them until they could be corroborated and the basis investigated. When we attempted to repeat them in 1937 and 1938 (during the early summer when fresh fruits were in abundance) we failed to obtain the same results. In fact it was not until the appearance of Case 4 in 1941 that the original findings were duplicated. Careful review of our original experiments indicated that the initial results followed the use

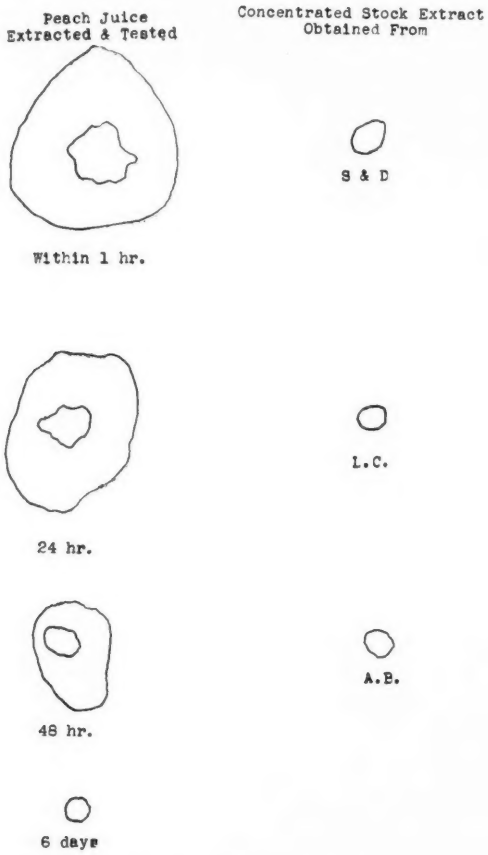

Fig. 3.—Direct skin tests on D. H.

of freshly extracted juice that had been used immediately in the skin tests, whereas in the later attempts on the same patients, the juices used had been prepared several days in advance of the time when the skin tests were performed. This naturally accounted for our inability in 1937 and 1938 to verify the initial observations. When we adhered to the original technique and tested with the freshly extracted juice, we were able to confirm our earlier findings both by direct skin reactions and passive transfer.

In order to determine the time required for the deterioration of the fresh juice to occur, freshly extracted juice was allowed to remain in the refrigerator and samples withdrawn at periodic intervals. Each sample was then tested both directly on the patient and in passively sensitized sites to ascertain the relative potency. The results illustrated in Figs. 3 and 4 demonstrate the rapid diminution in the potency of these extracts. Deterioration was greatest apparently within the first twenty-four hours and was complete within three to four days.

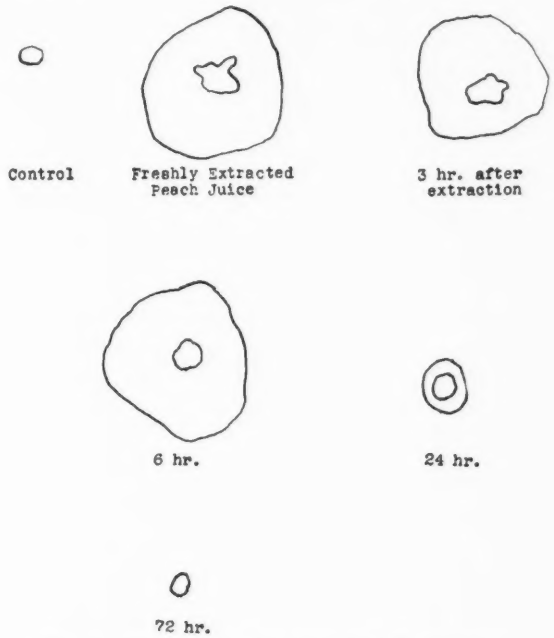

Fig. 4.—Passive transfer tests with D. H. serum.

It will be noted that these patients were able to take stewed or canned fruits with impunity. Evidently, therefore, the antigen must have been destroyed or altered in the process and skin tests with extracts of the denatured fruit were negative. We also were interested in the effect of rapid freezing (frozen fruits) upon the allergenic qualities of fresh fruits. Individual extracts were prepared from the stewed, canned, and frozen peaches in the same manner as from the fresh fruits. Direct and indirect skin tests with these extracts showed that the stewing and canning process rendered the extracts inactive while freezing did not destroy their allergenic properties (Fig. 5). The extracted juice of the frozen fruit deteriorated in the same manner and approximately at the same rate as that of the fresh fruit.

While attempting to sterilize the freshly extracted juices by Seitz filtration, it was noted that a marked diminution in activity occurred

(see Fig. 5). This procedure was repeated several times with identical results on each occasion.

Although the original direct skin tests were carried out with the fresh juices of those fruits to which the patients gave positive clinical histories, nearly all the subsequent observations herein reported were made with the peach extracts. We have suggestive evidence from other experiments that other fruit juices behave in a similar manner and hope to be able to include it in subsequent communications when the evidence is more definite and conclusive.

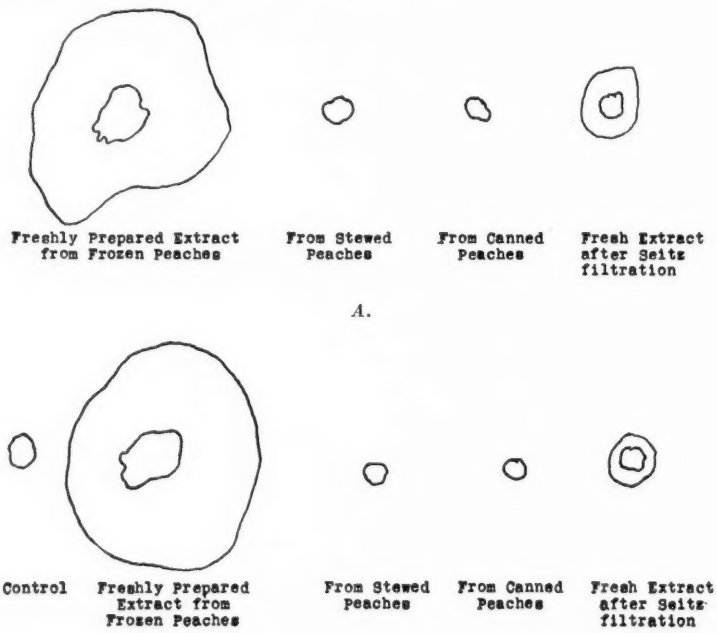

Fig. 5.—*A*, Direct skin tests on D. H.; *B*, Passive transfers with D. H. serum.

DISCUSSION

It is evident, therefore, from these results that the juices of fresh fruits, exemplified by the peach, contains a labile antigen which may be responsible for the clinical manifestations of fresh fruit sensitization. In the four patients studied, the symptoms produced by clinical trial with the offending fruit were quite similar and constant in each patient. Since skin tests with the stock extracts even in concentrated form were consistently negative, it was assumed that some change had taken place in the course of its preparation to render it impotent. This deterioration was indicated by the rapid diminution in the size of the skin test reactions obtained with the peach juice at various intervals after its extraction, even though it was kept in the refrigerator during this period.

Deterioration begins apparently within a few hours after extraction and may be complete within one to three days. The demonstration of the lability of the active agent in the peach juice upon standing explained our failure to verify our original observations made in 1936, because subsequent experiments were carried out with juice that was several days old. When we used the fresh juice shortly after extraction upon the same patients, positive reactions could again be obtained.

It will be noted both from the histories and the result of the skin tests with the fresh juice that each patient showed reactions to more than one fresh fruit but these were not always in the same botanical group. Thus Case 1 (E. L.) developed symptoms from peaches but not from plums, even though both are in the same botanical group. Further study will be necessary however in order to determine more definitely whether a common antigen is present in the various botanical groups of fruits.

The absence of positive skin reactions to the juices of stewed and canned fruits is in accord with the patients' clinical histories and with general clinical experience in food allergy. Denaturization of fresh fruits seems to alter or destory the allergenic factor in such a way as to enable patients clinically sensitive to the fresh fruit to ingest the denatured fruit without untoward effect. Rapid freezing on the other hand does not alter the allergenic property of the fresh fruits that we have thus far investigated.

Seitz filtration materially reduces the antigenic property of fresh fruit extracts. This raises the question of sterilization of these extracts by some other means that will not alter or reduce their allergenic properties. Other means of sterilization have been tried but have not yet been considered sufficiently satisfactory for present report.

SUMMARY

1. We have demonstrated the presence of a labile antigen in fresh fruit extracts. Deterioration is rapid, occurring in largest proportion within twenty-four hours and being complete within one to three days.

2. This antigen is either altered or destroyed by heat during both the canning and stewing processes.

3. Rapid freezing (as in frozen fruits) seems to preserve the allergenic properties of fresh fruits.

4. Seitz filtration materially reduces the potency of the freshly extracted juice.

5. The lability of the antigen in fresh fruit juice probably accounts for the frequent failure to obtain positive skin reactions in clinically sensitive patients even with the concentrated stock extracts.

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DISCUSSION

DR. LEO H. CRIEP.—This is a very timely subject. I suppose there is nothing that has helped to shake the confidence of the practicing physician in allergy more than these negative skin reactions that are obtained in clinically sensitive patients.

This is also a problem in which every allergist is interested because it deals with extracts, their preparation, preservation, and deterioration. Perhaps we could spend an afternoon some day in discussing the subject in order to arrive at some sort of standardization and uniformity. This is merely a pious wish.

Now, the authors have rendered us a very valuable service. They indicate that manipulation of materials frequently leads to loss of potency. For example, Seitz filtration, dialysis, and heating caused them to lose strength. That confirms the view that the less one fools with the material in preparing the extracts, the more likely it is that the extracts will be more potent. I certainly believe that is so.

The other point which Dr. Tuft and Dr. Blumstein emphasize, which is of particular interest to me, is the fact that freezing does not deteriorate the extract. We are now engaged in preparing extracts or at least in preserving them by drying through freezing, and our results have been very encouraging in that connection.

The pH of the extract, I think, is probably of more importance than we are accustomed to believe. For that reason, I often wonder to what extent phenol, which is used as a preservative by some of us, may interfere with keeping up the potency of the extract.

I remember one particular patient who had a rather unusual type of allergy manifested by vertigo, in whom I obtained a constitutional reaction by allowing a small amount of garlic to be introduced under his tongue. That patient gave a negative intradermal and a negative scratch test to garlic extract.

I often wonder whether the active principle which is lost is always a protein. Dr. Coca some time ago was subjecting some extracts to tryptic digestion and showed that they did not lose their potency.

DR. RACKEMANN.—Every once in a while something really important is presented to our meeting and I think this is one of those papers. I would like to congratulate Dr. Tuft and Dr. Blumstein on a fine piece of work.

DR. COOKE.—I merely wanted to add a word of commendation and verification of this in an experience which happened a good many years ago in a woman who had a very clear-cut history of reaction to raspberries, then in season. We tested her with our usual extract and got nothing at all. We then expressed the juice and immediately did a scratch test and got a very excellent positive reaction. She came in the following day and we again used the juice which had been prepared the day before, and to our amazement the test was negative. We confirmed the fact that a positive reaction could be obtained with the fresh juice of the raspberry but nothing further was done about it.

RESULTS OF INTRADERMAL SKIN TESTS WITH TRICHINA ANTIGEN IN ALLERGIC AND NORMAL INDIVIDUALS

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THE intradermal skin test for the diagnosis of trichinosis is not new. Bachman,[1] in 1928, was the first to give favorable reports on its diagnostic value. He used a saline extract of the dried larvae. Augustine and Theiler,[2] McCoy, Muller, and Friedlander,[3] Spink and Augustine,[4] Kaljus,[5] Bozicevich,[6] and McNaught, Beard, and Myers[7] all continued this work, confirmed it, and modified his technique of extraction in varying degrees. They all concluded that patients infested with Trichina gave immediate positive intradermal skin reactions eleven to forty-three days after infestation in 74 to 98 per cent of cases. This type of skin reaction was found to be positive in 6.7 per cent of normal controls in 47 cases studied by McCoy[3] and 6.4 per cent of 194 normals tested by McNaught and Anderson.[8]

In 1939, Baron and Brunner[9] studied eighteen atopic patients with skin tests of Trichina extract. All of these gave negative reactions at first. However, upon repeated testing a definite skin sensitization with the production of atopic reagins occurred. They concluded from this that the diagnostic value of intracutaneous tests diminished with their repetition in any single patient. However, Kaufman[10] as well as McNaught, Beard, and Myers[7] found that repeated tests with antigens did not provoke a positive skin reaction in a person *not* infected by Trichina.

In 1942, Witebsky, Wels, and Heide[11] developed a serodiagnostic complement fixation for trichinosis. They found that the antigen, when boiled for twenty minutes, was more specific than the unboiled extract. It was then decided to compare the boiled and unboiled extract by intradermal skin test on a person known to have a trichinosis infestation. As controls, by coincidence, two atopic individuals were chosen. There appeared to be no apparent difference between the two antigens in varying dilutions, and the control patients reacted just as strongly as the person known to have trichinosis.

In view of these findings and the discrepancies of skin testing with trichina antigen among various investigators, we then wondered whether

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Read at the Eighteenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., May 2, 1942.

this difference in results was due to the fact that one investigator used atopic patients and the others normal and whether association of an eosinophilia in atopic individuals and those having worm infestations had any significance in producing a positive skin reaction to the trichina antigen. It was decided, therefore, to compare the intradermal skin reactions of Trichina antigen in normal and allergic individuals.

METHODS AND MATERIALS

Two extracts of Trichina* were used in the following experiments. Both are saline extracts of the dried larvae, the former in a dilution of 1:8,000, and the latter in a dilution of 1:10,000. Any reaction in which the diameter of the wheal was greater than 0.3 cm. and the erythema greater than 1 cm. was considered a positive reaction.

Approximately 0.01 c.c. of each extract was injected intradermally on the forearm of the subjects tested. A control, using the extract fluid, was injected into a comparable skin site on the opposite arm. All glass tuberculin syringes were used throughout these experiments. The results of skin testing were recorded in twenty minutes. It was not possible to read these reactions again in twenty-four hours, as most patients were ambulatory outpatients.

TABLE I

COMPARISON OF INTRADERMAL TRICHINA SKIN TESTS WITH THE BOILED AND UNBOILED EXTRACTS IN TWENTY-EIGHT CONSECUTIVE ALLERGIC PATIENTS

NUMBER OF PATIENTS TESTED	ANTIGEN USED	MINIMAL POSITIVE REACTIONS*	PERCENTAGE OF POSITIVE REACTIONS	STRONGER POSITIVE REACTIONS†	PERCENTAGE OF POSITIVE REACTIONS
28	Unboiled	10	35	4	14
28	Boiled	9	32	4	14

*Considered a positive reaction if the average diameter of the wheal was greater than 0.3 cm. and the average diameter of the erythema was greater than 1 cm.

†Considered a positive reaction if the average diameter of the wheal was greater than 0.4 cm. and the average diameter of the erythema was greater than 1.5 cm.

RESULTS

1. Comparison of Boiled and Unboiled Antigens.—Twenty-eight consecutive patients in the allergy clinic were skin tested with 0.01 c.c. of both the boiled extract (boiled for twenty minutes) and the unboiled extract. The results were read and recorded in fifteen to twenty minutes. As seen in Table I, ten patients gave positive reactions to the unboiled antigen and of these nine also reacted positively to the boiled antigen. There was no apparent difference in the size of the reactions. Unfortunately, in this group no control tests were used, but in all subsequent experiments the extracting fluid as provided with the Eli Lilly test was used as a control.

*The United States Public Health Service, through the courtesy of Dr. W. Wright, supplied us with one, and the Eli Lilly Co., the other.

2. Comparison of the Eli Lilly Antigen (1:10,000) and the United States Public Health Service Antigen (1:8,000) in Patients in the Allergy Clinic.—Ninety-one consecutive patients were given simultaneously intradermal skin tests with both extracts and a saline control (Table II). Twenty-two gave positive reactions to the U.S.P.H.S. extract, but eight of these had positive controls; that is, they reacted to the buffered saline solution as well as the antigen. Therefore, only fourteen of eighty-three, or 16.9 per cent, reacted positively.

Twenty patients of this same group gave positive reactions to the Lilly extract but seven of this group reacted positively to the saline control. Therefore, only thirteen of a final eighty-four, or 15.4 per cent, were positive.

TABLE II

COMPARISON OF ELI LILLY EXTRACT (1:10,000) AND UNITED STATES PUBLIC HEALTH SERVICE EXTRACT (1:8,000) IN ALLERGIC PATIENTS

NUMBER OF PATIENTS ORIGINALLY TESTED	EXTRACT USED	NUMBER OF REACTIONS TO SALINE CONTROL	NUMBER OF PATIENTS FINALLY AVAILABLE	NUMBER OF POSITIVE REACTIONS TO EXTRACT ALONE	PERCENTAGE OF POSITIVE REACTIONS
91	U.S.P.H.S.	8	83	14	16.9
91	Lilly	7	84	13	15.4
91 + 22 (113)	Lilly	9	104	20	19.1
113	Lilly U.S.P.H.	16	97	22	22.6

An additional twenty allergic patients were tested with the Lilly antigen and control alone. Of this group, seven gave positive reactions, but there were two positive reactions to the saline control. Therefore, of all 104 allergic patients tested with Trichina extract (Lilly), twenty, or 19.1 per cent, gave positive reactions.

An interesting fact was that some of these individuals reacted to one extract and not the other, and vice versa, there being no regular difference in reactions between them.

In total of all allergic patients tested, 113 in all, thirty-eight gave positive reactions to either one or the other extract. However, sixteen had positive reactions to the saline controls. Therefore, twenty-two of ninety-seven finally were available for study, or 22.6 per cent, were true positive reactions.

3. Comparison of the Eli Lilly Antigen (1:10,000) and the United States Public Health Service Antigen (1:8,000) in Patients in the General Medical Clinic.—One hundred and fourteen consecutive patients in the general medical wards and out-patient department of the Buffalo General Hospital were tested in the same way as the allergic group, using both antigens and control (Table III).

TABLE III

COMPARISON OF ELI LILLY EXTRACT (1:10,000) AND UNITED STATES PUBLIC HEALTH SERVICE EXTRACT (1:8,000) IN NORMAL INDIVIDUALS

NUMBER OF PATIENTS ORIGINALLY TESTED	EXTRACT USED	NUMBER OF REACTIONS TO SALINE CONTROL	NUMBER OF PATIENTS FINALLY AVAILABLE	NUMBER OF POSITIVE REACTIONS TO EXTRACT ALONE	PERCENTAGE OF POSITIVE REACTIONS
114	U.S.P.H.S.	5	109	7	6.5
114	Lilly	2	112	4	3.3
114	U.S.P.H.S. or/and Lilly	5	109	8	7.4

Twelve of these gave positive reactions to the U.S.P.H.S. extract, but five gave positive reactions to the saline controls. Therefore, seven of 108, or 6.5 per cent, were positive to the extract alone.

Six reacted positively to the Lilly antigen. Two of these, however, gave positive reactions to the control; therefore, four of 112, or 3.7 per cent, were positive.

In total, using the results of both antigens in all patients, eight of 108, or 7.4 per cent, gave a positive reaction to one or the other extract.

4. Comparison of all Skin Reactions in the Allergic Group.—The group of patients tested with Trichina antigen in our allergy clinic had been previously skin tested with the routine inhalants, epidermals, pollens, and foods. The percentage of all inhalant factors to which the patients gave positive intradermal reactions was determined, as was the percentage of positive reactions to the foods. The incidence of positive reactions was then compared between subjects in the group which reacted to Trichina extract and the subjects in the group that did not.

Those who gave positive reactions to either Trichina antigen and negative controls had previously reacted to 43 per cent of the inhalants and 33 per cent of the foods; whereas, those allergic patients who did not react to the Trichina extract reacted to only 26 per cent of the inhalants and 22 per cent of the foods.

TABLE IV

COMPARISON OF SKIN REACTIVITY WITH ALL ALLERGENS TO THE REACTIVITY OF THE POSITIVE AND NEGATIVE TRICHINA TESTS

REACTION TO TRICHINA EXTRACT	PERCENTAGE REACTION TO INHALANTS	PERCENTAGE REACTION TO FOODS
Positive with negative controls	43	33
Negative	26	22

5. Effect of Repeated Skin Tests With Trichina Extract on Allergic Individuals.—Twenty-five consecutive patients in our allergy clinic were tested with the same extract (Lilly) in the same manner two to three

weeks apart. Seven of these patients showed an increase in the size of the reaction on retesting. One was negative on the first test but definitely positive on the second test. Seven patients did not react on either occasion. Eleven of the twenty-five showed a definite decrease in the size of the reaction.

DISCUSSION

The incidence of Trichina infestation in human beings as determined by autopsy findings has varied with many investigators throughout the country as anywhere between 3.5 to 36 per cent, the average being about 17 per cent. Recently, Most and Helpon[12] found 22 per cent infestation of human diaphragms at autopsy in New York City. McNaught and associates found 24 per cent of 200 diaphragms infested. In testing 194 normal individuals, he found that 6.7 per cent gave an immediate reaction and 18.1 per cent gave a delayed twenty-four-hour reaction. He believed that this delayed reaction was evidence of past infestation. Therefore, summing up the immediate and delayed reactions, his results compared quite favorably to the autopsy findings. It must be remembered that the same individuals who were skin tested were not necessarily the ones autopsied.

In our series of normal patients we found that 7.4 per cent reacted positively to Trichina antigen, which compares quite favorably with that of other investigations, as shown in Table V.

TABLE V

RESULTS OF SKIN TESTING NORMAL INDIVIDUALS WITH TRICHINA EXTRACTS

INVESTIGATORS	NUMBER OF PATIENTS	PERCENTAGE OF POSITIVE REACTIONS
McCoy	47	6.4
McNaught, Beard, and Myers	113	7.4
Arbesman, Witebsky, Osgood	194	6.7

However, of our allergic group, 22.6 per cent gave definitely positive reactions. None of these patients had any symptoms of trichinosis or a definite past history of these symptoms. This does not, nevertheless, exclude the possibility that they may have had the disease in the past in some subclinical form, but it is unlikely to assume that the incidence of trichinosis in an allergic group is greater than a normal group. There was no correlation in this group of sensitivity to pork or beef protein. These positive reactors had a highly reactive skin, as evidenced by the great percentage of positive reactions to other substances. If one could assume that the atopic or allergic individual had the peculiar characteristic of retaining his ability to produce an immediate reaction and not a delayed reaction, our percentage of positive skin tests to Trichina antigen in allergic individuals would compare very favorably with the incidence found at autopsy.

Baron and Brunner[9] showed that this test was not specific for Trichina extract alone. *Ascaris lumbricoides* was found to give even stronger reactions to the reagins produced by the Trichina. Other investigators likewise found positive skin reactions to Trichina with other worm infestations. There is an eosinophilia in worm infestations and also in atopic individuals. However, we could find no correlation between the percentage of an eosinophilia and the positive skin reactions. Likewise, McNaught and his co-workers found eight to ten patients who gave negative reactions who also had an eosinophilia of 51 to 65 per cent, whereas, seventeen of eighty-three patients who had an eosinophilia of 3 per cent or less gave positive skin reactions to Trichina extract.

Although twenty-five of our allergic patients were retested with Trichina extract, we could find but one patient who reacted positively the second time who failed to react the first time. For routine skin testing with these dilutions, we agree with McNaught and Kaufman that there is no apparent change of reactions upon retesting on persons not infested with Trichina. However, Baron and Brunner used considerably stronger, larger, and more frequent intradermal injections to develop this sensitization.

One can readily see the difficulty in differential diagnosis of a patient with angioneurotic edema of the eyelids, general malaise, and an eosinophilia in an atopic patient, as compared to the early symptoms of trichinosis. Certainly a positive intradermal skin test with Trichina antigen would be of little help here.

Inasmuch as this intradermal skin test does not become positive, often until six weeks after infestation, one must frequently repeat tests, three to four times, if found negative at first in a suspected case. Thus, one must keep in mind the fact that a false positive reaction may develop as the result of repeated skin testing. The same mechanism also takes place when testing for undulant fever. Several previous intradermal tests may lead not only to false skin tests later on but even to a false complement fixation or serologic test. A more specific aid is the recent complement fixation test with Trichina antigen.[11] They found only one false positive reaction in one thousand patients who were not having symptoms of the disease.

This is a preliminary report. We hope to do this complement fixation test on all the patients, both allergic and nonallergic, who gave positive skin reactions to the Trichina antigen, and also to continue retesting these patients.

SUMMARY

1. Two groups of patients were skin tested with Trichina antigens. The normal group reacted in 7.4 per cent of the cases and the allergic group in 22.6 per cent.

2. Of the allergic group, those who reacted positively also reacted to a greater percentage of the other allergens, 43 per cent of inhalants, and 33 per cent of the foods, whereas the nonreactors gave positive reactions to only 26 per cent of the inhalants and 22 per cent of the foods.

3. The possibility of false positive reactions, latent previous infestations, or an excess of H-substance in the skin of allergic individuals must be considered in interpreting these tests.

4. The trichinosis skin test as a diagnostic procedure in normal and more particularly in allergic individuals is of definitely limited importance for the diagnosis of recent infestation.

5. However, a negative reaction in an allergic individual, provided the disease is of five to six weeks' duration, might be of significance in ruling out trichinosis.

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DISCUSSION

DR. FINEMAN.—We have used the Trichina extract as a great aid in the diagnosis of trichinosis. In the past year at Sydenham Hospital we have had on the wards three patients in whom we suspected this disease. The intradermal test was employed in 1:10,000 dilution, and marked local whealing was observed. We then did passive transfer studies and were able to demonstrate reagins in high dilution. Samples of blood were sent to Dr. Witebsky who reported the presence of complement fixation. These findings aided us in making the diagnosis

of trichinosis. Muscle biopsy later showed the presence of encysted larvae. One can use this diagnostic skin test with great help, particularly in cases where one suspects the possibility of Trichina infestation.

DR. ARBESMAN.—I want to bring out the point that a positive skin reaction is not the final answer to anything, particularly in allergic individuals. Likewise, a positive skin reaction to Trichina antigens with no other manifestations of the disease must be carefully evaluated.

There is only one other thing. We did not do passive transfer tests on these allergic individuals who gave positive reactions, because we wished to compare the antigens as given to the general practitioner for the diagnosis of trichinosis and see how valuable it would or would not be.

ALLERGY IN IDENTICAL TWINS

Report of Seven Pairs of Twins

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THIS is a report of seven pairs of identical twins showing allergic manifestations. A review of the literature indicates some previous similar references. Cooke and Vanderveer[1] reported in 1916 six pairs of allergic twins. Only three pairs in their series showed similar sensitizations. Further casual reference to the occurrence of allergy in identical twins is to be found in a general discussion on heredity in allergy.[2] In this discussion Benson cites two pairs, Fineman one pair, and Credille one pair of identical twins with exactly similar allergies. Cohen refers to "several" sets of twins and Kahn to one pair of twins in which only one showed evidences of allergy. Simon[3] reports one pair of identical twins, one of whom had asthma more severely than the other. Spaich and Ostertag[4] studied the occurrence of allergic manifestations in a large series of twins. They point out that allergic disorders when present in one twin will frequently occur in the other.

The following report will emphasize: (1) nature of twins (whether identical or heterozygotic twins); (2) allergic diagnosis; (3) the age of onset; (4) family history of allergy; (5) specific allergens causing sensitivity history, direct skin tests, and passive transfer; and (6) course of the disease.

CASE REPORTS

Pair 1.—Jean and Jane N., 7 years of age. These two little girls, identical twins, were first seen in 1938 when their stepmother gave us similar histories for each. It was noticed that at the age of 4 years they presented nasal and ophthalmic manifestations such as rhinorrhea, lacrimation, and itchiness of the eyes. They had acquired a pony and began to develop symptoms several weeks after playing with the animal. The pony was sold and the children did well for about two years when they moved to a farm where they were again exposed to animals and to various types of vegetations. Shortly afterward both children manifested nocturnal asthma and some hay fever, although one's symptoms were not very marked. The father had asthma and hay fever. Paternal grandmother had bronchial asthma.

Intradermal tests showed in both children marked or moderate reactions to the following: horse dander, horse serum, pyrethrum, glue, cattle dander, dust, tobacco, pork, milk, string beans, peanuts, hazelnuts, and ragweed. Passive transfer tests performed with the serum of each child indicated the presence of reagins in each

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serum to ragweed, dust, horse dander, pork, and milk. Clinical trial proved the existence in each of sensitivity to animal danders and of allergy to pork and ragweed. There were a few slight and some doubtful reactions to some vegetables, fruits, and other substances, although these tests were not found to be the same in both twins. However, those reactions that were definitely marked and proved by passive transfer were identical in both. Each of the twins improved following general allergic treatment. Only Jean needed ragweed therapy. They were discharged in 1941.

These two children present clinical evidence (asthma) of sensitivity to horse dander and symptoms following the ingestion of pork and milk at exactly the same age and presumably after the same amount of contact. While only one child developed marked clinical ragweed hay fever, it is to be noted that both showed the presence of reagins against ragweed pollen.

PAIR 2.—Helen and Hilda N., 23 years of age. These two young women, identical twins, were first seen in June, 1941, presenting a long-standing history of bronchial asthma. Their father states that both children began to be bothered with paroxysms of asthma at about the age of 4 years and "almost on the same day." The condition has continued to be present continuously since then, increasing in severity and accompanied by periods of rhinorrhea and sneezing, usually worse in June. The symptoms are identical in both girls except that Hilda's condition is more severe than Helen's. The father has asthma. The patients live on a farm. Both have noticed on numerous occasions that ingestion of bananas and strawberries will initiate an attack of asthma. Helen suspects allergy to onions. Environmental change causes improvement in both patients.

Intradermal tests yield marked and moderate reactions in both patients to animal danders (cattle, chicken), flaxseed, dust, kapok, milk, garlic, soybean, huckleberry, and nuts; positive passive transfer was obtained with the sera of both patients to all of the above with the exception of soybean and huckleberry. Hilda showed a negative reaction to intradermal testing with onion extract. There were two or three instances in which Helen reacted moderately to some substances (vegetables and spices) when no skin reaction was shown by Hilda. Both girls yielded a positive skin reaction and positive passive transfer with grass (timothy) pollen extract; only Helen, whose grass hay fever is more severe, could be persuaded to take pollen treatments. They continue to live in the country in separate homes (Helen is married), and their condition is somewhat improved.

The similarity of manifestations in this pair is striking. The date of onset of bronchial asthma was, to quote the father, "almost on the same day." Both patients volunteered the information that certain foods, bananas and strawberries, brought on an attack. Here again there is a pointed similarity in the marked positive skin tests and positive transfers.

PAIR 3.—Marie and Helen K., 44 years of age. These identical twins were first seen in December, 1940, at which time they complained of paroxysms of asthma. Marie states her attacks were much more severe and started at the age of 9 years, practically "at the same time as her sister's." There was no seasonal history. The sister's attacks (Helen's) became less and less severe, making it unnecessary

for her to seek medical consultation at this time. However, she submitted to an allergic survey at our request. Marie shows evidence of paranasal sinus infection and a beginning bronchiectasis. She states that for as long as she could remember she would break out in hives and begin to wheeze on eating onions, an experience which was shared by her sister. Both patients were markedly sensitive to house dust. The mother and a maternal uncle had hay fever.

Intradermal tests yield marked and moderately positive reactions to house dust, orris root, and eggs in Marie, and less marked but positive reactions to these in Helen. Positive passive transfer was obtained to dust and orris root with the sera of both and to eggs with the serum of Marie. Indeterminate and unrelated mild positive reactions were obtained to quite a few other substances without positive passive transfer and without clinical evidence of sensitivity in this connection.

Both patients showed negative intradermal tests even to a concentrated extract of onion, but Marie showed a positive scratch test performed with onion juice. This gave a negative reaction in Helen. Marie's condition has been improving under treatment.

This pair presents the same factors of interest as Pair 2. The onset occurred almost at the same time. Both patients were allergic, although one lost her clinical manifestations while the other continued to get worse and developed bronchiectasis. It should be noted that the latter had severe infection in the sinuses which complication may explain the continuation and severity of her asthmatic condition.

PAIR 4.—Edward and Milton C., 14 years of age.* These are identical twins. Edward was first seen in 1940 at the age of 12 years, presenting a history of late hay fever and seasonal asthma. Positive intradermal reactions were obtained to mixed ragweed pollen extract and to grasses. There is no allied allergy. His brother Milton was seen at the age of 13 years, when he developed severe asthma following exposure to paint. In addition to perennial asthma he presents symptoms of grass hay fever and yielded positive intradermal reactions to timothy and plantain. One year later this twin gave good positive intradermal reactions to ragweed, although he did not evidence any symptoms during the ragweed hay fever season. He also shows marked reactions to several inhalants and foods. The maternal uncle has asthma and the mother has severe migraine.

Each of these twins had hay fever, one twin being allergic to grasses and the other to ragweed. It should be noted, however, that positive skin reactions were obtained in both patients to ragweed and grasses.

It is true that one twin (Milton) presented himself one year later than Edward, at a time when, following exposure to paint, he developed symptoms severe enough to warrant his seeking medical aid. The question naturally arises as to whether the irritation of paint was not merely a trigger mechanism, accentuating a condition which was present but unnoticed for some time previously. It may be possible that this twin may have had some hay fever symptoms at the same time that his brother, but that these were so mild as to pass by unrecognized.

*Reported through the courtesy of Dr. Sylvia Wechsler.

PAIR 5.—John and Richard B., 9 years of age. These identical twins were seen two years apart. John was observed at the age of 7 years, when he presented a flexor eczema which came on at the age of 3 years. Richard was first seen two years later, when he was 9 years old, and presented a mild dermatitis of the face, neck, and flexor surfaces, the onset of which was at about the age of 4 years. The mother states that John's condition would be aggravated by the ingestion of eggs; that on at least two occasions he developed asthma following eating eggs. She suspects that Richard is sensitive to both eggs and wheat. Neither twin showed any allied allergies. Their father has asthma.

Intradermal and passive transfer tests were definitely positive to eggs in each twin. In addition John showed such positive reactions to dust and buckwheat; Richard to wheat. John yielded only positive intradermal reactions to dog, silk, wool and several vegetables, fruits and nuts. Richard showed positive skin tests to milk, chocolate, nuts and fish.

Both twins had allergic eczema and present definite evidence of sensitivity to eggs. One was allergic to wheat and the other to buckwheat. The similarities are striking, although the age of onset is slightly different.

PAIR 6.—Majorie K., 33 years of age. This patient has had bronchial asthma since she was 16 years old. There is no allied allergy. She is clinically sensitive to house dust (musty places), feathers, and peanuts. Her mother had asthma and a cousin had hay fever. Strong positive intradermal tests were obtained with dust, orris root, feathers, milk, peanuts, and fungi. Positive passive transfer was obtained to dust, feathers, and fungi.

She states that her sister, an identical twin, developed asthma at the age of 18 years. She recalls her sister to have been markedly sensitive to house dust and "animals." Following a severe attack of asthma, her sister developed pneumonia and died at the age of 27 years. With allergic management this patient's condition improved considerably.

No studies were carried out on one of the twins. The history is again significant. There is a strong family history of allergy, and clinical manifestations were identical. However, the date of onset was slightly different.

PAIR 7.—Judith and Trudy K., 23 months of age. These two babies, identical twins, the daughters of a physician, showed (according to their father) a typical urticarial reaction at the age of 8 months when they were first given 1 gr. of aspirin for a cold. The reaction came on approximately two or three hours after the administration of the drug. There were no asthmatic manifestations. It was noticed subsequently that each and every time the infants would receive a small dose of aspirin or any other coal tar derivative, such as salicylate, the same reaction would occur. It is also stated that when the babies were first given eggs they both developed a mild eczema on the flexor surfaces of the elbows. This reaction kept up for a period of about two or three months, but since then both children developed a tolerance for eggs. An aunt on the paternal side has hay fever and the grandfather on the paternal side has asthma.

Intradermal tests are positive (moderate) in both twins to egg extract, this reaction persisting even at the time when both can eat eggs without showing symptoms.

These two babies present identical allergies to drugs (aspirin) and eggs, showing the same clinical manifestations whose onset was exactly on the same date.

DISCUSSION

It is significant that the patients reported in this paper present without exception a strong positive family history of allergy. The date of onset of the allergic disorder was, with slight variation, about the same in each of the pairs of twins. To some extent even the allergies were similar; that is, in many instances each of the twins was allergic to the same substances. Where there was evidence of pollen allergy, the same pollen was not always the offending one in each twin, but each showed the presence of reagins against both pollens. The severity of the symptoms was usually slightly different and in some instances one of the twins came in for study only at special request. In those instances in which only one twin reported for treatment, it is to be noted that the continuation of symptoms in this twin was invariably due to such complicating factors as sinusitis and bronchiectasis. Such differences as may appear were only slight and could be accounted for by minor variations in exposure. Furthermore, the absence of symptoms in one twin need not necessarily indicate an absence of allergy in this individual as shown in the report by Vaughan.[5] He cites a pair of twins, one having urticaria and the other having allergic manifestations. Both were exposed to diphtheria and received diphtheria antitoxin. The twin who showed no allergy died as a result of serum sensitivity. This twin was allergic, although this was not known.

An excellent summary of the literature indicating analogous similarities in the occurrence of various other medical conditions in identical twins is given by Margolis and Eisenstein,[6] who report about the brothers cited by Trousseau, one of whom wrote his twin in a distant city, "I am now having my rheumatic ophthalmia, you must be having yours."

These data are of interest from both a genetic and allergic viewpoint. It leads to the tempting conclusion that a constitutional factor or an allergic tendency must be transmitted through heredity, that is, through the germ plasm (chromosomes).

If it were possible by this means to establish definitely the presence of such identical conditions in two individuals derived from the same ovum, this might prove of even greater significance than the large percentage of positive family history of allergy obtained in patients with allergic disorders.

Identical twins belong to the same sex and are very much alike. They are derived from the fertilization of the same ovum and therefore obtain their hereditary characteristics from the same chromosomes. This may explain the marked similarity in the allergies presented by the twins reported in the series. The tendency toward the development of allergic

disorders is even more marked than the constitutional diathesis which has been shown to play an important role in many other diseases such as, for example, diabetes, cancer, etc. It cannot be denied, however, that environmental factors play an equally important role in patients so constituted by heredity. Note, for example, the similarity of sensitivity to the same allergens (horse dander, eggs, pollen, pork), in each set of twins due presumably to similar exposure or contact. And yet, it would be difficult to explain the development of specific sensitivity on the basis of exposure alone, for the twins reported in this paper show no evidence of allergy to many common allergens, substances to which they had been exposed constantly. Identical twins form ideal subjects for a study of this kind not only because they have similar heredity, but also because they are brought up under practically similar environmental conditions.

SUMMARY

1. Case reports of seven pairs of identical allergic twins are presented, including direct and passive transfer tests on each.

2. These reports emphasize the following: (A) the similarities in allergic manifestations in identical twins; (B) the marked constitutional tendencies towards the development of allergic conditions in each; (C) occasional slight differences which may be explained by minor variations in exposure and contact.

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MAY BUILDING, TENTH FLOOR.

DISCUSSION

DR. SELIAN HEBALD.—My only qualification for discussing this paper is that I have happened to observe one set of allergic identical twins.

In respect to the point of similarity of sensitivity, that is, their being reactive to the same substances, they were very similar to the ones that Dr. Criep told you about, but in respect to the point of onset, the date of onset, they were very different. One developed hay fever that lasted all summer at the age of 4 years. His hay fever continued for several years. Seven years later his brother developed ragweed hay fever and 9 years later, timothy hay fever.

The one who developed hay fever first has asthma rather severely, and as a result of that, now at the age of 24 years, he is about two inches shorter and ten pounds lighter than his identical twin.

It is difficult to account for this difference in the dates of onset, because they were inseparable. The environment certainly was grossly identical. The genetic material that they inherited was, of course, identical.

There is a geneticist by the name of Goldsmith who has speculated on a possible mechanism based on hereditary differences in a dislocation or a translocation of the particles or molecules of which genetic material is made. Whether or not that is an adequate explanation of these differences, I am unable to answer.

DR. A. H. FINEMAN.—I have had occasion to observe three sets of identical twins, and my observations were quite similar to those of Dr. Criep. In each instance, the sensitivities were the same for both children and the age of onset of the allergies was the same. As a matter of fact, when one child developed an attack of asthma, the other did. The sensitivities in one set were wheat, oat, mustard, radish, ragweed and dust. These children had asthma and later developed ragweed hay fever in the same year.

DR. WARREN VAUGHAN.—We have had two sets of twins who looked very much alike who had different allergic manifestations. In the more recently tested set, one twin has atopic dermatitis and the other has hay fever, and they do not react to the same allergens.

Several cases previously reported in the literature have been reviewed and some do not have the same symptoms or react in the same manner.

When twins look alike, they are not necessarily identical twins. Prof. Horatio Newman of the University of Chicago has done a great deal of work on the physiology of twinning and has made the statement that even though they may look almost exactly alike, it takes a real expert to tell whether they are truly uni-oval twins. Now, this fact may explain some of the differences in the literature and in the example which I have just described in one of our sets of twins. I presume that the most satisfactory procedure would be to have competent geneticists work with the allergists in any such studies. I would like to make one suggestion to Dr. Criep or to anyone else in the room who might have the opportunity. I have often thought that it would be fun to attend a twin convention and get the twins interested in allergy and to see what could be found. You have all seen pictures of these twin conventions in the rotogravures.

Newman has gone to the twin conventions in just the same way that a lot of people follow the circuses. He has found and described in his book several series of identical twins who were separated almost at birth and never met until many years later. By the law of chance, some of these may be allergic. It would be especially interesting to make a study of these groups to determine whether different environments influence sensitization.

DR. W. C. SPAIN.—There is so much historical medicine bandied back and forth in this meeting that I might say to Dr. Criep that in the Inquiry Into the Human Faculty, Sir Francis Galton, over 100 years ago, describes a pair of what he thought were identical twins. To prove that they were identical he stated that every time they went into a certain home both would develop asthma and at the selfsame time. Probably these twins were dust-sensitive cases.

Now, I would like to ask Dr. Criep just what his criteria are for establishing the fact that these are identical twins. I think it was eighteen or nineteen years ago that Dr. Cooke and I had had a group of some thirteen or fourteen pairs of twins upon whom we had worked up case histories, family histories, tests and practically all data for publication, in fact, in completed manuscript form, when we went over to see Dr. Stockard, who was at that time the head of the Department of Anatomy at Cornell. By the time we were through with that interview, we were completely crushed. We decided not to publish our work. Dr. Stockard told us that there was no way of proving that twins were identical except by the examination of their fingerprints. The fingerprints of one had to be the mirror images of the other. Of course we could not obtain these data. I would like to know if Dr. Criep has done any investigation of this sort.

DR. CRIEP.—Dr. Stull sprang this on me as I walked into the hall. He said, "Did you get fingerprints on your twins?" and the answer was "No."

However, most of these twins are available and I will do that. In general, I was told that it was safe to assume that twins were identical if they look exactly alike and if they are of the same sex. They have physical characteristics which are almost indistinguishable. Now, that probably is scientifically not adequate.

I omitted references to literature, but Dr. Cooke reported in 1916 six pairs of twins, three of which had identical allergies. And now, I may also say just before leaving Pittsburgh I received a communication from Dr. Forman of the International Allergy Correspondence Club and in that communication he acquaints me with the fact that he has gathered, as a result of a questionnaire, a total of almost 100 pairs of twins who had allergies and that information may be of some further value in connection with our discussion.

I was also referred to the President of the International Twins Association for further information on the subject.

THE TREATMENT OF RHUS POISONING BY ALCOHOLIC EXTRACTS IN A SMALL GROUP CONTROLLED BY PRELIMINARY PATCH TESTS

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RHUS dermatitis was first described by Captain John Smith of Pocohantas fame in 1609. He described the plant and also the rash which resulted from contact in human beings. Others, Cornutus in 1635, Linnaeus in 1736, and Peter Kalm in 1748, agreed that the plant was of poisonous character. Bigelow in 1820 suggested that the various specimens were probably varieties of the same species, *Rhus toxicodendron*. Botanists have disagreed many times on the proper classification of the Rhus family. Certainly the difference is not great and is of purely academic interest rather than of practical importance.[1]

Methods ranging from the oral administration of portions of the plant in its raw form (leaves, etc., or extracts made from the plant and administered as a tincture or triturate) to hypodermic injections of the extract in a multiplicity of vehicles all have been used. The results are somewhat at variance, but of course the methods used differed widely.

The present study was directed toward the use of Rhus preparations as a prophylactic measure in controlling dermatitis. We were confronted with the problem of protecting, if possible, a group of boys 14 to 18 years of age, who were to spend the greater part of the summer of 1941 in a mountain camp where Rhus was known to flourish. Rhus dermatitis had been the scourge of the camp year after year. Not uncommonly, boys were confined to bed in the small infirmary for as long as ten days. A few of them had had repeated admissions for the same cause in a single season. The camp directors, moreover, had planned during 1941 to clear off the underbrush from some additional land. The boys participating in this work were, therefore, more exposed to poison ivy than they had ever been.

An alcoholic extract prepared by Spain,[2] according to methods previously outlined, was used. Each of the boys was given a preliminary patch test, using a 1:500 or 1:100 dilution of this extract. If there was a history of dermatitis, a 1:500 dilution was used in the patch test. If there was no history of previous dermatitis, 1:100 was used. Patch testing was done on April 29, 1941, and observed May 2, 1941.

The extract used for injections was diluted in Coca's fluid by placing an accurately measured amount of the concentrated extract into an

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accurately measured amount of diluent, so that 0.5 c.c. of the resultant mixture was equivalent to 0.05 c.c. of the dilutions given in the following schedule. Each member of the group was given five subcutaneous injections at weekly intervals, as follows:

0.05 c.c. of 1:100 dilution on May 6
0.05 c.c. of 1:50 dilution on May 13
0.05 c.c. of 1:10 dilution on May 19
0.05 c.c. of concentrate on May 26
0.05 c.c. of concentrate on June 2

No additional injections were given to any member of the group.

The boys went to camp on July 22. They were requested to report any skin eruption to the resident physician promptly. There was considerable scepticism among them about the worth of the treatment, and the authors are convinced such reports were made rather gleefully and without hesitation on their parts. Suffice it to say, no member of the group was confined to bed because of Rhus dermatitis.

One hundred and seven boys received patch tests and injections, but complete data were obtained on only eighty-two of the group. Eleven of the eighty-two had no cutaneous reaction to the patch test. Seventy-one boys, or 86 per cent, had a positive reaction to the patch test. All of the boys were given prophylactic injections. Seven of them had the rash as severely or more severely than in previous years. Nineteen had a less intense or trifling eruption, and fifty-six escaped entirely. In 8.5 per cent the prophylaxis was an apparent failure.

The entire group has been subdivided into Group A, those who had had poison ivy in former years, of whom thirty-four (or 98 per cent) had positive patch tests, and Group B, or those who had never had poison ivy, of whom forty-two (or 88 per cent) had positive patch tests.

In Group A there were thirty-five individuals. Seven of these had the rash as severely as in former years. Twelve of them had a less severe, or a mild eruption, and sixteen escaped the rash entirely. The treatment was an apparent failure in 20 per cent of this group.

In Group B there were forty-seven individuals who had never had poison ivy dermatitis. Seven of the total group had a very mild eruption. The percentage of total failure was, therefore, 0.

In this series there were no constitutional reactions. There were three individuals who developed mild eruptions while receiving the prophylactic treatment. Two boys had a small patch on the arm in which the injection was given. It was felt that a drop of material might have been accidentally spilled on the affected areas. Another boy developed a few small spots on his face, possibly from the same source. No other eruptions or untoward reactions occurred in any member of the group.

Many other authors who have used hypodermic methods for prophylaxis report no more serious difficulty. Several have reported dermatitis (22.2 per cent) in persons who had received hypodermic injections. It was felt that the initial dose given these individuals was too large and the difficulty could be obviated by giving smaller amounts and gradually increasing the doses. Templeton,[3] in 1929, reported localized or generalized urticaria in four individuals who had received injections of Rhus toxin. He, however, did not state the vehicle employed nor did he give the concentration of the extract used other than a trade name which cannot be identified. No other investigator has observed urticaria as a reaction to Rhus. It seems more likely that the urticaria was either accidental or a reaction to the vehicle used, quite likely an oil. All in all, the danger of constitutional symptoms from Rhus injections are few and far between and certainly a much less factor than they are in pollen injections.

Stomatitis,[4] pruritus ani and perianal dermatitis,[5] and eruption around the urethra have occurred in those who have taken the plant or an extract of it by mouth. These very annoying symptoms do not occur when the material is injected.

TABLE I

RESULTS OF PROPHYLACTIC TREATMENT OF POISON IVY DERMATITIS IN A GROUP OF EIGHTY-TWO BOYS 14 TO 18 YEARS OF AGE

Whole Group	
Boys treated	82
Rash as bad or worse than previous years	7
Rash less intense or trifling	19
Escaped entirely	56
Apparent failures (per cent)	8.5
Group A	
Those who had had poison ivy in former years	35
Rash as severe as formerly	7
Rash less severe	12
Escaped entirely	16
Apparent failures (per cent)	20
Group B	
Those who had never had poison ivy	47
Rash—mild	7
Escaped entirely	40
Apparent failures (per cent)	0

Reports on the prophylactic treatment of Rhus dermatitis as a whole have been fairly satisfactory. The methods used and the results obtained by various investigators are shown in Table II. Only two observers have reported less than 50 per cent good results. By far, the majority have found that 75 per cent or more of the patients have been definitely benefited. Generally speaking, the percentage of good results is higher if four or more injections are given. The method of administration of the Rhus toxin seems to make little difference. There-

TABLE II

COMBINED RESULTS OF TREATMENT WITH RHUS TOXICODENDRON

AUTHOR	NUMBER OF PATIENTS	NUMBER OF INJECTIONS	EXTRACTING FLUID	VEHICLE	METHOD OF ADMINISTRATION	RESULTS (PER CENT)	PATCH TEST	YEAR REPORTED
Dakin[5]					Leaves orally			1829
Allembaugh[6]			Alcohol	Alcohol	Tincture orally	Good		1898
Schamberg[7]			Alcohol	Water	Hypo and orally			1919
Strickler[8]	20	4	Alcohol	Water	Hypo and orally	90		1923
Bivings[9]	15	8	Alcohol	Water	Hypo and orally	87	No	1924
Spain and Cooke[2]	98	10+	Absolute alcohol	Alcohol	Hypo and orally	95	Yes	1927
Gowen[10]	3	1	Ether	Almond oil	Hypo	100	No	1933
Blank and Coca[11]	95	4	Acetone	Olive oil	Hypo	86	No	1936
Molitch and Poliakoff[12]	40	10+	Alcohol	Alcohol	Hypo	100	Yes	1936
Molitch and Poliakoff[13]	42	10+	Alcohol	Alcohol	Hypo	100		1937
Caulfeild[14]	4	10+	Ether	Corn oil	Hypo	75	Yes	1937
Bachmann[15]	14	2	Acetone	Almond oil	Hypo	15		1938
Biederman[16]	13	10+	Alcohol	Physiologic saline	Hypo	77		1938
Zisserman and Birch[17]	304	1 or 2	Ether	Olive oil	Hypo	69 40		1939
Greenberg and Mallozzi[18]	77	2	Acetone	Almond oil	Hypo	73	Yes	1940
Shelmire[19]	40	None	Ether	Alcohol	Orally	100	Yes	1941
Zisserman[20]	153	15-30	Ether	Tablets	Orally	57	No	1941
Gold and Masucci[21]	20	Large doses	Ether	Tablets	Orally	85	Yes	1942

fore, the choice of preparation and the method of administration would seem to depend upon the individual who is to receive it and the physician who is to give it.

SUMMARY

1. We feel that the prophylactic treatment consisting of hypodermic injections of alcoholic extract of *Rhus toxicodendron* have been quite satisfactory.

2. The material given in gradually increasing doses did not cause any generalized eruption or any local discomfort.

3. The annoying pruritus ani and perianal dermatitis were entirely lacking.

4. No member of the group was incapacitated because of Rhus dermatitis.

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DISCUSSION

DR. LESLIE N. GAY.—At one of the first meetings of the Society for the Study of Asthma and Allied Conditions, in 1926, Dr. Spain and Dr. Cooke presented the first publication that pertained to the treatment and diagnosis of *Rhus toxicodendron* dermatitis. They prepared a 95 per cent alcoholic extract of the dried leaf. Because oxidation set in quickly, they finally arrived at the conclusion that the small amount of water in the extract caused precipitation of some agent which inactivated the material. Therefore, they hit upon the idea of extracting with absolute alcohol and have managed to maintain a rather permanent material. However, it has been our experience that the ether extract prepared by Dr. Caulfeild is a more stable and certainly a more potent preparation than the absolute alcohol extract.

There are two outstanding pieces of work which I think deserve mention. One is the publication of Heinbecker, who found that of sixty-five Eskimos patch tested with the extract of *Rhus toxicodendron* not one gave a reaction, and he concluded therefore that contact with the material is necessary before one can consider an individual sensitized. Later, Strauss patch tested a number of infants with poison ivy extract and found that they failed to react. Then he patch tested them again after three or four weeks and, much to his surprise, discovered that a large proportion of them, 72 per cent, gave a positive patch test.

This demonstrated definitely that contact with the material was necessary before sensitivity could be demonstrated.

In our clinic at the Johns Hopkins Hospital, the alcoholic extract prepared according to the technique of Cooke and Spain has been heretofore used with excellent results. In 1931, twenty patients between the ages of 4 and 38 years were admitted with moderately severe lesions due to poison ivy. Subcutaneous injections brought about rapid improvement of the symptoms, relieving especially the itching. Daily injections were administered and in every case after the third dose a most satisfactory therapeutic result was recorded. However, from the recent observations of the results from the ether extract it is my impression that we have a more stable and more active material for the diagnosis and treatment of *Rhus toxicodendron* dermatitis.

DR. GRAFTON TYLER BROWN.—It is an interesting observation that some of the commercial poison ivy preparations vary greatly in potency. Some years ago, Dr. Coca supplied me with some concentrated acetone extract, a 13 per cent acetone extract I believe, and we prepared various dilutions in corn oil from that, and patch tested a series of patients with poison ivy. At the same time we did parallel patch tests with all of the therapeutic or prophylactic ivy extracts that were commercially available. Some of them are quite potent and others are completely inert. In other words, the latter would not produce any skin reaction at all on highly sensitive patients. Certainly no therapeutic results could be expected, therefore, to be obtained from their use.

Another interesting observation that Dr. Coca passed on to me is that he felt the acetone itself had some therapeutic value. There were some patients in the clinic who were treated erroneously with acetone in oil, and there was no ivy at all in the preparation. They got excellent coseasonal results from it, in fact even a shade better than with the ivy extracts themselves. So, we have to evaluate all of these cases with a good deal of caution.

DR. HERMAN GOLD.—First, I would like to know whether a control series was available during 1941 to compare the incidence of poison ivy among the treated and untreated groups; second, a few years ago, following the late Dr. Caulfeild's presentation on the use of ether extracts, I treated a rather large group of patients sensitive to poison ivy with a very potent ether extract of poison ivy. The patients were given weekly subcutaneous injections over a period of two to three months, and in many the final dose reached was 1 c.c. of a 1:50 dilution. A decrease in the skin sensitivity, as judged by patch tests, was noted in some patients, but upon exposure to poison ivy (severe field test), I got exactly 100 per cent failure in both the treated and the control groups. So, I think that we have to be extremely careful in accepting successful results in poison ivy prophylaxis unless the clinical experiments are well controlled.

DR. HANNA.—In reply to Dr. Gold's question, I might state that we did not have a control group. We were called upon by the physician in charge of a school for boys in the city of Philadelphia. This group of boys were going to clean up the underbrush in a camp in the Poconos. They had previously had a great deal of trouble with poison ivy. Their infirmary had been full all summer long with boys who had the eruption very seriously. This particular group of boys were the ones who were delegated to do the clean-up job.

I visited the camp and know that there was a great deal of poison ivy there. As I say, we have no report of a control group.

ORAL PROPHYLAXIS AGAINST POISON IVY

Follow-Up Report

Herman Gold, M.D., and Peter Masucci, B.S.
Glenolden, Pa.

In a previous communication[1] we reported our experience with the oral method of prophylaxis against poison ivy. With the dose schedule and tablet preparation used in the first group of twenty patients (Group A), sensitive to poison ivy, we found that seventeen of them responded favorably to treatment while three did not. The nature of the response, whether favorable or not, was determined by skin patch tests and controlled field exposure to poison ivy performed one to three weeks after prophylactic therapy had been terminated. The three failures could not be ascribed to inadequate dosage because all of the patients received 3 Gm. or more of the poison ivy oleoresin over a period of forty-five days. In addition to a decrease in skin sensitivity to poison ivy, the daily administration by mouth of the oleoresin-containing tablets produced a marked increase in intestinal and systemic tolerance to the drug. Thus the initial dose was 0.5 mg., while the final dose was 150 mg. daily, and one patient actually ingested as much as 500 mg. of the oleoresin without any complaints. It is true that in the course of development of this tolerance, 80 per cent of the patients developed toxic skin reactions that varied from localized or diffuse erythema, either punctate or solid, to large vesiculation, particularly of the fingers, with edema and desquamation. These skin reactions occurred most often in the first two to three weeks of treatment and were most likely an expression of overburned tolerance due to too rapid an increase in dosage. Interestingly enough, if treatment was continued in the presence of these skin reactions, the patients managed to tolerate slowly increasing doses with total clearing of the skin. Thus the end and total dosage of oleoresin was about the same in all the patients.

Naturally, this rather frequent occurrence of skin reactions would possibly limit the value of this method of desensitization against poison ivy. In order to determine whether it was possible to eliminate these untoward reactions, we treated a second group of thirteen patients (Group B) sensitive to poison ivy with the same tablets over a period of sixty days, as shown in Table I. The initial dose was the same as in Group A, one 0.5 mg. tablet, but the dosage was increased more

From the Medical Research Division, Sharp and Dohme, Inc.

Read at the Eighteenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., May 2, 1942.

TABLE I

PROPHYLACTIC ORAL THERAPY AGAINST POISON IVY

DAILY INTAKE

DAY OF TREATMENT	NUMBER OF TABLETS	MG.	DAY OF TREATMENT	NUMBER OF TABLETS	MG.
1	1	0.5	30	2	10.0
2	1	0.5	31	2	10.0
3	1	0.5	32	2	10.0
4	2	0.5	33	3	10.0
5	2	0.5	34	3	10.0
6	2	0.5	35	4	10.0
7	3	0.5	36	4	10.0
8	3	0.5	37	2	25.0
9	3	0.5	38	2	25.0
10	1	2.0	39	2	25.0
11	1	2.0		1	10.0
12	1	2.0	40	2	25.0
13	2	2.0		2	10.0
14	2	2.0	41	3	25.0
15	2	2.0	42	4	25.0
16	3	2.0	43	4	25.0
17	3	2.0	44	4	25.0
18	3	2.0	45	4	25.0
19	4	2.0	46	4	25.0
20	4	2.0	47	4	25.0
21	4	2.0	48	4	25.0
22	1	10.0	49	4	25.0
23	1	10.0	50	4	25.0
24	1	10.0	51	5	25.0
25	1	10.0	52	5	25.0
	1	2.0	53	5	25.0
26	1	10.0	54	5	25.0
	1	2.0	55	5	25.0
27	1	10.0	56	5	25.0
	2	2.0	57	5	25.0
28	1	10.0	58	6	25.0
	3	2.0	59	6	25.0
29	1	10.0	60	6	25.0
	4	2.0			

TABLE II

DAILY INTAKE

DAY OF TREATMENT	GROUP A (MG.)	GROUP B (MG.)
First	0.5	0.5
Third	2.0	0.5
Seventh	8.0	1.5
Tenth	10.0	2.0
Fifteenth	12.0	4.0
Twenty-first	50.0	8.0
Thirtieth	100.0	20.0
Forty-fifth	150.0	100.0
Sixtieth	–	150.0

slowly, as shown in Table II. Notwithstanding this slower schedule, the results were disappointing. Treatment had to be discontinued in one patient after ten days because of a severe bullous eruption of the hands and feet; four patients developed rather severe erythemas of the extremities and four other subjects complained of mild and

transient erythema of the arms and legs. Pruritus was an accompanying symptom. Again these toxic signs occurred in most cases in the first two or three weeks of treatment. It would be possible, perhaps, to eliminate these skin reactions by spreading out the oral therapy over a still longer period of time, four to five months, which would allow a much smaller initial dose, such as 0.1 or even 0.05 mg. tablet and a more gradual daily increase in the oleoresin ingested. However, such a prolonged type of therapy would not be practical unless the desensitization that results therefrom is of considerable duration. To throw some light on this phase of the problem, we performed skin patch tests in the Spring of 1942 on the patients in Group B, six months after the completion of treatment, and on nineteen members of Group A about ten months after the last patch and field tests had been done. The technique described in our previous report[1] was employed in the performance of the patch tests. Two 1:100 oil dilutions of the *Rhus toxicodendron* were applied on opposite forearms. Solution No. 1 was the same preparation we had used in the Spring of 1941. It had been kept in a corked bottle at room temperature for about a year. Solution No. 2 was freshly prepared before use from the same stock concentrate as solution No. 1. The comparative readings showed that solution No. 2 was a little stronger than solution No. 1, although the latter retained a great deal of its potency. Deterioration was apparently slight.

The results of the patch tests in Group A were as follows: Of sixteen patients that had favorably responded to oral therapy, four showed a definite increase in skin sensitivity, seven showed a slight increase and five showed no change from the tests performed ten months before. A further analysis of the results of these patch tests in reference to those performed for control purposes before oral therapy was started showed that five patients had returned to the control level of sensitivity while eleven of them still retained some evidence of decreased reactivity to poison ivy. Among the five that had returned to the control level of sensitivity, there were three patients whom treatment had rendered nonreactive to poison ivy (1:100 patch test). The other three members of Group A had failed to respond to oral therapy, and, upon retesting, they showed no change in their skin reaction (+++, vesiculation with edema).

Patch tests performed on the patients in Group B six months after treatment had been completed showed the following: two patients gave a + reaction (mottled erythema), four patients showed a ++ reaction (solid erythema with edema), and seven patients gave a +++ reaction (vesiculation). Of five control subjects, two gave a + reaction and three showed a +++ reaction. The results of treatment in Group B as judged by the patch tests were definitely inferior to those obtained in Group A. Since the original sensitivity was about the same in both

groups, the explanation for their difference in response to oral therapy may be found in the interval of time that elapsed between completion of treatment and the performance of the patch tests which in Group B was six months as compared to one to two weeks in Group A. This explanation gains further support from the results obtained on repatch testing of Group A ten months after treatment. It would, therefore, appear that the skin desensitization that results from oral therapy against poison ivy is not of long duration, lasting in some patients only a few months.

SUMMARY

1. Thirty-three patients sensitive to poison ivy were given prophylactic oral therapy in the Spring and Summer of 1941. The dose, reactions and results of follow-up patch tests are described.

2. The incidence of toxic skin reactions was high, and the desensitization obtained appears to be of rather short duration, lasting in some patients only a few months.

REFERENCE

1. Gold, H., and Masucci, P.: Prophylactic Oral Therapy Against Poison Ivy, J. ALLERGY 13: 157, 1942.

DISCUSSION

DR. J. A. CLARKE, JR.—The treatment of poison ivy is rather peculiar. It is very old and runs back over a great many years. In fact, we found a Dr. Dakin in New Jersey who suggested treatment by mouth as early as 1820.

In the table that Dr. Hanna showed, practically every investigator who attempted treatment has reported success, and yet you will notice the greatest variation in the type of treatment. One man used only two injections. This simple treatment was almost as good, apparently, as treatment consisting of a great number of injections. Certainly, the number of injections and the mode of administration seem to make comparatively little difference in the results of the treatment.

Possibly, our standards for successful treatment are too high. If we would put every hay fever patient down as a failure who gave two successive sneezes every week, we would not brag about what we did for hay fever. Yet the tendency is to regard any poison ivy treatment as an absolute failure if a man had a little patch on his finger after he touches a leaf.

As a matter of fact, all of the patients whom we have treated, who were severely sensitive, received a very satisfactory result. This does not mean that they do not have little patches on their fingers once in a while or on their arms or legs, but they are not laid up in bed with the severe generalized dermatitis that they have had in other years.

The choice between subcutaneous injections and feeding the weed by mouth, to my mind, lies very decidedly in favor of the hypodermic treatment.

In our group of patients, as Dr. Hanna told you, we had only three untoward reactions and they could, all of them, very easily have

resulted from the spilling of a little bit of the ivy extract either on the arm or face, careless technique in other words. We had no unusually severe local reactions, and we had no rashes appearing anywhere else on the body.

Everyone who has fed the poison ivy by mouth has reported a certain amount of pruritus ani or an actual dermatitis around the anus, which, to my mind, constitutes a very serious objection to that form of treatment.

There are, of course, true failures—those who have the rash as severely as they did in years without treatment. It is possible that these are due to plants or weeds other than those in the Rhus family. Or, just as we have found that there are various proteins or fractions in ragweed pollen, each capable of producing hay fever in certain sensitive persons, so there may be various fractions of the toxic principle of the Rhus plant. All of these may not be extracted in our present technique.

I think we should all advise the prophylactic treatment of Rhus dermatitis, particularly in those persons who have a very severe clinical reaction on exposure to the weed. We can save a great many days of illness in this group.

On the other hand, those who come in with just a small localized patch on an arm or a leg, it is not my custom to advise them to go through a course of treatment.

DR. GOLD.—In Group A we judged success of treatment by evidence of decreased skin sensitivity as determined by patch tests. In other words, if a patient gave a +++ patch reaction to a 1:100 extract before oral therapy and then gave a ± reaction to the same extract when retested one to two weeks after treatment was finished, we considered this a favorable response.

In addition, we further tested the clinical results of treatment by field exposure to poison ivy. Both treated and untreated patients were taken into a poison ivy field. The plants were rubbed into the skin. The results of this exposure in both groups were then compared. In the follow-up study we found that the favorable response to oral therapy lasted about five to six months, as judged by the results of patch tests. Thereafter there was a tendency for the skin sensitivity to poison ivy to return to the original level found prior to treatment.

THE FINANCIAL AND SOME SOCIAL ASPECTS OF ASTHMA IN THE CLINIC PATIENT

G. E. Gaillard, M.D.
New York, N. Y.

DURING the course of investigation and treatment of asthmatic patients who applied to the Postgraduate Allergy Clinics, personal interviews were obtained from over 600 patients. This work comprised a review of the social status of the patients, the expenses pertinent to their illnesses, and generally the social aspects of the asthma problem.

The Postgraduate Hospital is situated on the lower east side of New York City. It is in a district populated by people in the more modest income brackets and from this group draws a moderate percentage of its patients. The clinic, however, is not subject to zoning regulations, so that somewhat more than 50 per cent of its attendance is derived from distant sectors of the city and suburbs.

The study was made upon the patients seen in both the pediatric and adult allergy clinics. The combined attendance of these clinics has averaged over 20,000 patient visits per year for the period under study, 1935-1940.

The clinics are operated on a free and pay basis. About 20 per cent of the patients are attended without charge. The remainder pay moderate fees. Theoretically, no patients are treated who are above the income group of clinic patients.

The data of this investigation are offered in tabulated form.

The cases in Table I are about equally divided between male and female. There are slightly more female than male.

In each of the age groups from 1 to 19 years and over 60 years there were 65 per cent males and 35 per cent females.

In the age group from 20 to 60 years there are 65 per cent females and 35 per cent males.

There is no explanation and no precedent for the preponderance in attendance of male patients in the young and older age groups.

The preponderance of female patients in the middle age groups is understandable since in this age group, the bulk of employable males would not be able to attend day clinics.

From the Medical Department, Allergy Division, New York Postgraduate Medical School and Hospital, Columbia University.

Read at the Eighteenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, May 2, 1942.

TABLE I

PATIENTS CLASSIFIED BY AGE AND SEX

AGE GROUPS (YR.)	MALE	FEMALE	TOTAL	PER CENT
1 to 9	81	38	119	19.4
10 to 19	49	33	82	13.3
20 to 29	41	60	161	16.4
30 to 49	72	144	216	35.0
50 to 59	23	32	55	9.0
60 and over	24	14	38	6.21
Age not given	5	0	5	0.8
Total	295	321	616	
Per cent	48	52		

TABLE II

PATIENTS CLASSIFIED BY NATIVITY

NATIVITY	MALE	FEMALE	TOTAL	PER CENT
Born in U.S.A.	72	56	128	20.8
Born in U.S.A. (One or both parents foreign born)	135	140	275	44.4
Foreign born	86	122	208	34.0
Nativity not recorded	2	3	5	0.8
Total	295	321	616	

TABLE III

PATIENTS CLASSIFIED BY RELIGION

RELIGION	MALE	FEMALE	TOTAL	PER CENT
Jewish	116	140	256	41.6
Catholic	125	126	251	40.8
Protestant	53	54	107	17.2
Mohammedan		1	1	0.4
Not stated	1		1	
Total	295	321	616	

TABLE IV

PATIENTS CLASSIFIED BY STOCK NATIVITY

STOCK NATIVITY	MALE	FEMALE	TOTAL	PER CENT
Italian				
Born in Italy	22	25	121	19.8
Born in U.S.A. (One or both parents born in Italy)	37	37		
Jewish				
Born in Europe	40	59	254	41.2
Born in U.S.A. (Parents born in Europe)	64	67		
Born in U.S.A. (Parents born in U.S.A.)	11	12		
All Others				
Born abroad	24	38	62	9.8
Born in U.S.A. (Parents foreign born)	33	36	69	11.0
Born in U.S.A. (Parents born in U.S.A.)	61	44	105	17.4
Not recorded	2	3	5	0.8
Total	295	321	616	

Tables II, III, and IV need no further elucidation. It is understood that factors controlling the nativity and religion of patients would undoubtedly vary according to the location in which the study was made. This study probably gives a fairly accurate cross-section of the paying clinic clientele of the borough of Manhattan.

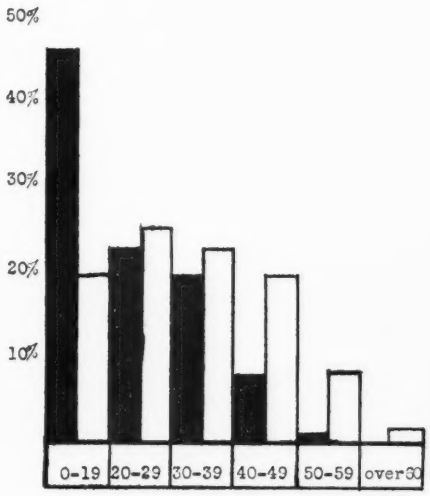

Fig. 1.—Sensitive group.

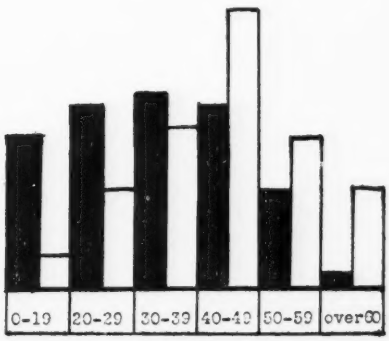

Fig. 2.—Infective group.

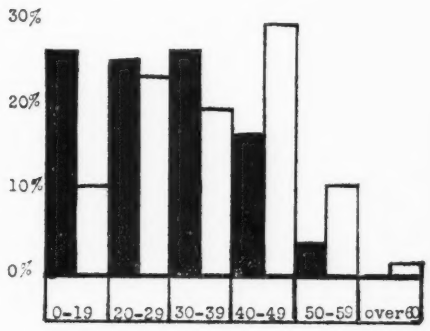

Fig. 3.—Mixed group.

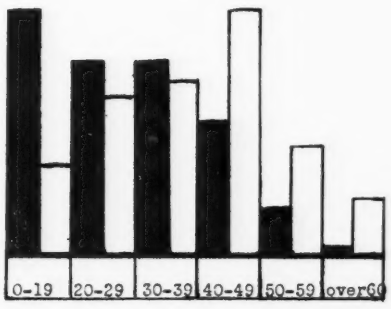

Fig. 4.—All groups.

The Jewish population of New York City* by the latest census is about 30 per cent of the total population of the city. Over 40 per cent of the patients in this study were Jewish.

Figs. 1, 2, 3, and 4 are composite diagrams showing the percentage of patients in each age group for the age of onset of their illness and the age at which they first attended the clinic. The shaded bars represent the age at onset and the unshaded bars the age at which the clinic was first attended.

*Figures from the Jewish Statistical Bureau, 181 Broadway, New York, N. Y.

For purposes of diagnosis, treatment, and correlation of the asthmatic patients treated at the postgraduate clinics, the cases as far as possible are divided into three diagnostic groups: sensitive, infective, and mixed. The sensitive group is that in which the asthma is due wholly to extrinsic factors such as inhalants or ingestants. The infective group is that in which there is no evidence of extrinsic sensitivity, and in which there is a demonstrable infection, usually of the upper respiratory tract, considered to be responsible for the asthma. The mixed group is that in which both sensitive or extrinsic, and infective elements are thought to be the etiologic agents. Obviously, it is not possible definitely to classify every patient, but patients in whom the causative factors could not be proved were classified with the group with which they seemed to be identified.

Fig. 4 is the group chart of all of the cases studied while the other three charts represent the cases according to their etiologic classification.

Examination of the shaded bars, representing the age of onset of the asthma, in Fig. 4 reveals that of the cases reviewed, 75 per cent had the onset of asthma during the first four decades. In the first two decades, along with the third and fourth, about an equal number of people were afflicted.

In the sensitive group (Fig. 1) 90 per cent of the patients had asthma by the end of the fourth decade. Indeed, almost half of all the skin-sensitive cases had developed their clinical symptoms by the age of 20 years, and practically all of them by the age of 40 years. In the infective group (Fig. 2) only 63 per cent had the onset during this age span. In other words, only one-fifth of all the infective cases had developed clinical symptoms by the age of 20 years, and by the age of 40 years one-third had yet to acquire their asthma. Of the asthmatic patients in the mixed group, 80 per cent developed their illness during these first four decades.

Examination of the unshaded bars, representing the age at which the patients first attended the clinic, reveals, in general, a rising tendency in the first four decades as compared to a falling tendency in the shaded bars. In Fig. 4, while 75 per cent of the patients began their asthma before the age of 40 years, only 51 per cent sought assistance during this while. However, by the age of 50 years, 80 per cent of all the cases had attended the clinic. This tendency to a lag of a decade between the age of onset and application for therapy of an equivalent percentage of patients seems to follow in the other three figures. It is especially well shown in Fig. 1. Here, 47 per cent of the patients had the onset in the first two decades, while it was three decades before 46 per cent applied for treatment. Again in this figure another 23 per cent began their asthma in the third decade, while in the fourth decade, 23 per cent first attended the clinic. Again in the fourth decade, an-

other 20 per cent began their illness, while 20 per cent more applied for treatment in the fifth decade. Similar percentages hold essentially true for the other figures. It would seem then that of the patients studied, there was a lapse of about ten years from the onset of their illness until treatment was solicited at this clinic.

The facts brought out in these figures should be of prime interest. In general as the bronchi become more sensitized and infected and the asthma more deeply seated, the treatment becomes more difficult. The lag between the appearance of symptoms and the seeking of assistance may be explained partly as due to the widespread but most often erroneous impression that an asthmatic patient can outgrow his asthma. The asthmatic patient as well as the medical profession should realize the fallacy of this frequently given advice. Assistance should be sought as soon as possible after the onset of clinical symptoms while the condition is still reversible and before the development of secondary pathologic changes.

TABLE V

ALLERGIC CONDITIONS ASSOCIATED WITH ASTHMA

GROUP	HAY FEVER	URTICARIA	ECZEMA	MIGRAINE	ANGIONEUROTIC EDEMA	GASTROINTESTINAL ALLERGY	DRUG ALLERGY	TOTAL	NO ASSOCIATED CONDITION	PER CENT OF PATIENTS WITH ASSOCIATED CONDITIONS
Sensitive	121	16	19	3	2	2	1	165	32	65
Infective	5	7	5	1				18	159	10
Mixed	57	7	11	1		1		77	51	60
Undiagnosed	2		1					3	93	3

In Table V allergic conditions associated with the asthma are considered. Here it may be noted that, as may be expected, the percentage of patients with associated allergic conditions is highest in the sensitive and mixed groups. In the infective group of asthmatic patients, only 10 per cent had associated allergic conditions.

TABLE VI

FORMER PATIENTS CLASSIFIED WITH RESPECT TO ATTENDANCE
REASONS GIVEN FOR NOT ATTENDING

	MALE	FEMALE	TOTAL
Not now attending	27	45	72
"Cured"	8	14	
Not improving	13	9	
Private treatment	4	6	
Financial	3	11	
Time inconvenient	2	1	
Cannot commute	2	6	

Table VI classifies the patients with respect to their attendance to the clinic. Of the 616 cases reviewed, seventy-two, or 11.6 per cent,

have discontinued treatment for various reasons. Of this group, however, only twenty-two patients, or 4 per cent, of the total number have discontinued attendance because they reported no improvement from the treatment. This group was balanced by the twenty-two patients who have discontinued treatment because they felt they were cured. The remainder of the patients terminated the treatments for the various reasons listed.

TABLE VII

PATIENTS REPORTING IMPROVEMENT AND NO IMPROVEMENT

	MALE	FEMALE	TOTAL
Reported Improvement			
Attending	196	207	
Not attending	15	31	
Total	211	238	449
Reported No Improvement			
Attending	21	19	
Not attending	11	13	
Total	32	32	64
			513

Table VII lists the patients according to the results which they reported from their treatments. In this group of 513 patients, sixty-four, or 12 per cent, of the total reported no improvement from the treatment. However, only twenty-four patients or less than 5 per cent of the total number discontinued treatment because they were not improving. These figures were obtained from the records of patients who had attended the clinic for several months or more so that the tally should be reasonably accurate.

Because of the great number of patients attended and the variable personal equations on the staffs of clinics, it is difficult to obtain an accurate idea of the results of treatments to clinic patients. These tables may give at least an indirect appraisal of the benefits from therapy, which the patients derive at the postgraduate asthma clinic. Since the patients are from the small income group, and the clinic is essentially a pay clinic, the feeling is that the patients would discontinue treatment unless they felt they were receiving some help.

The fact that over a period of several years only 12 per cent of the patients felt they were not being helped and that less than 12 per cent of the patients discontinued their treatments for a variety of reasons makes us feel that the benefits of the clinic to the public are definite.

Of the patients attending the clinic about 40 per cent were employable of whom 12.3 per cent were unemployed. The latter condition was probably due to the general economic situation as well as to reasons pertaining to health. The remaining 60 per cent were housewives, children, and students who would not ordinarily be occupied by remunerative pursuits.

TABLE VIII

WORKING—NOT WORKING

	HOUSE-WIVES	STUDENTS OR PRE-SCHOOL	EMPLOYED	UNEM-PLOYED	TOTAL
Total	167	204	166	77	614
Per cent	27.3	33.2	27.2	12.3	

It is apparent that an economic survey of this group must be approached from two angles: (1) the monetary economic loss due to illness of the employables and (2) the difficulties imposed by ill health upon those people in nonremunerative occupations.

TABLE IX

AVERAGE MONEY SPENT PER PATIENT PER YEAR ON MEDICAL CARE

	MEDICINE ($)	PATENT MEDICINE ($)	CLINIC FEE ($)	OTHER HOSPITAL BILLS ($)	DOCTOR BILLS ($)	TOTAL
Sensitive						
Adult	4.50	1.15	17.50	1.00	11.80	35.90
Pediatric	2.83	0.16	13.16	0.96	10.32	28.03
Infective						
Adult	15.80	2.50	16.80	3.20	30.00	68.30
Pediatric	1.85	--	12.05	1.71	8.21	23.82
Mixed						
Adult	8.90	1.00	15.60	1.60	9.80	36.90
Pediatric	1.93	--	14.06	3.36	4.35	23.70

Table IX deals with the cash outlay incurred by all of the patients in the care of their asthma. Of these, the largest outlay per patient, $68.30, was made by the adult infective asthmatic patients who from experience we would expect to be the more ill. The second largest outlays of $36 and $37 were made by the adult sensitive and mixed asthmatic patients. The smallest outlays were made by those in the pediatric group among whom the cost varied little according to the classification of their illness. The greater part of the bulk of the difference between the costs of the disease in the adults and children was made by the difference in the outlay for medicine, hospital, and doctor bills. These facts would point to better results in children from the treatment which as a general rule is the finding in asthma and allergic diseases.

While the adults as a whole spent more money per capita on medicines than did the children, it is interesting to note the rather wide discrepancy between the money spent by these two groups on patent medicines. Patent medicine for this study was construed to mean self-prescribed medication. While the entire outlay for patent medicine among the adults averaged roughly $1.50 per patient, that of the pediatric group averaged only five cents. This discrepancy may be due to the education of the younger parents to seek medical advice

and care, rather than to rely upon advertised panaceas, or due to hesitancy upon the part of the young parent to administer medication without advice.

It is encouraging to note the difference between the outlays for prescribed and patent medications in the adult group. Of the averaged total money spent for medicine, only 13 per cent was for patent medicine.

The greatest amount of money spent for medicine is among the infective group which is to be expected because of the more tenacious character of the illness. This group spent about $18.30 per person for medicine as compared to $9.90 by the mixed asthmatic patient and $5.65 by the sensitive asthmatic patient.

The money spent for other hospital bills, including laboratory work and hospitalization, varied according to the etiology of the asthma. No account was taken in this study for the benefits which may have been derived by the patients from hospitalization insurance or from social service. Of the adults, the infective asthmatic patients spent about twice as much as the mixed and three times as much as the sensitive cases. It is our experience that the largest group of social service cases is among the severely ill infective cases. If the money spent by social service were included in the survey, it is likely that the money spent on the infective patients for hospitalization would be proportionately much greater. There is no reasonable explanation for the relatively large amount of money spent by the mixed cases of asthma of the pediatric group for hospital bills.

The quantity of money spent by the patients in clinic fees is about the same for all groups in their respective clinics. This situation is what one would suspect, since the fees vary but little according to the number of visits per patient per year. The cost of the clinic to the adults averages $16.60, and that of the children about $13 per patient per year.

An interesting expense and one which is unexpectedly high is that of outside doctor bills. While the money spent among the adult sensitive and mixed cases was less than for the clinic fees, the money spent by the adult infective group was considerably greater than the clinic fee. The total money spent by this group was almost as great for doctor bills as it was for clinic fees. The money spent by the children for outside doctor fees, while less than that of the adults, was still a large proportion of the total money expended in the care of their illness.

Table X is a breakdown of the expenditures listed in Table IX to reveal the percentage consumed by each item of the total money spent. In reading this table, it must be remembered that the total outlay of money by the infective group was almost as great as the combined

TABLE X

UNIT PERCENTAGE OF MONEY SPENT FOR MEDICAL CARE

	MEDICINE (%)	OTHER HOSPITAL BILLS (%)	CLINIC FEE (%)	DOCTOR BILLS (%)
Adult				
Sensitive	16	2	49	33
Infective	27	5	24	44
Mixed	27	4	42	27
Average	23	3	38	36
Pediatric				
Sensitive	10	4	46	40
Infective	7	7	50	36
Mixed	8	13	58	21
Average	8	8	52	32

outlays of the sensitive and mixed groups among the adults. Thus, while the clinic fees for all three groups are about the same, the percentage of the total spent by the infective cases for clinic care is only about one-half of that spent by the other two groups.

The relative amount of money spent by adults on medicine is almost three times that spent on the children. Here again is brought out the relation between the clinic fee and the fee to outside doctors. Among the adult group the total money spent to outside doctors was almost as great as the clinic fee. The fee to outside doctors was almost twice the clinic fee among the infective asthmatic patients. These two expenses account for about 75 per cent of the total outlay per patient. While medical attention accounts for over 80 per cent of the total outlay of money for the children, about one-third of it is the fee to home physicians.

A survey was made to determine the economic loss by disability of the patients in this group. Of the 134 employed people who reported disability, the average weekly wage was $21.15. The total number of days lost both complete and partial was 1,597 for a period of one year. The average number of days lost per person was about eleven. Thus, computing on a six-day week, the money lost per head per year was $38.70.

This estimate, of course, does not take into account the time lost by housewives and students, nor would there be any way to determine the economic value of such time lost.

It is understood that such economic loss is subject to variations according to the type of work done by the employed individual. The degree of asthma which could force a complete layoff for a laborer might cause only partial disability in the case of a worker in a sedentary occupation. Thus, people in higher income brackets would probably be relatively less affected economically than those in lower brackets where the percentage of laborers would be greater. Of course, there is no way to estimate the economic loss to employers caused by the

host of asthmatic patients who although ill were able to report for duty and to whom there was no economic penalty.

It is very probable that the relative economic loss caused by asthma is higher in the lower income bracket groups. It must be understood, however, that any deductions must be cautiously made because of the great number of variables to be considered.

SUMMARY

A survey on the financial and some social aspects of asthma was made upon 616 patients in the postgraduate hospital allergy clinics.

1. The cases were about equally divided between the sexes.
2. Ninety per cent of the sensitive cases developed their symptoms before the age of 40 years. Sixty-three per cent of the infective cases developed their asthma before the age of 40 years. There is an apparent lag of about a decade between the onset of clinical symptoms and the solicitation of treatment.
3. About 40 per cent of the clinic clientele is Jewish. The Jewish population of New York City is about 30 per cent of the total population.
4. Over a period of several years only 12 per cent of the patients felt that they did not improve with treatment. Only 4 per cent discontinued treatment because they were not improving.
5. The average money spent per patient per year for the treatment of the asthma in all groups was $36.10. The average money spent per person in the adult infective group was $68.30.
6. An account of the money spent for medical care is as follows:
   A. Adult asthmatics: 75 per cent was for medical service (clinic and home physician); about one-half of this amount was the fee to home physicians.
   B. Adult infective asthmatic patients: 68 per cent was for medical service; about two-thirds, or 44 per cent, of this was the fee to home physicians.
   C. Children: 84 per cent was for medical service; about three-eighths of this was the fee to home physicians.
7. Of the employed people who reported disability, the average number of days lost per year was eleven or about two full working weeks.

Acknowledgment is made to Dr. W. C. Spain and Dr. Robert Chobot for their cooperation in supplying the material from which these data were derived and for their advice in correlating the data.

DISCUSSION

DR. RACKEMANN.—Dr. Gaillard has collected many very interesting figures and two or three of them need emphasis. In the first place, the lag of ten years between the onset of asthma and the time of beginning treatment is interesting. He and I went over my chart to see what I could show about this lag between the onset and the time of the visit. We found out in this little series of fifty cases that the lag was 8.2 years, which compares very well with his lag of ten years.

The lag is easily explained, I think. No doubt these people have tried other things before they come to us. What we would like to know is whether their asthma was always as severe as it appears to be when they first come to us. That, of course, is hard to evaluate.

Dr. Gaillard found that the infective group had other allergy in 10 per cent of the cases. This goes along with the idea of a fundamental background which explains not only asthma but other symptoms as well.

As for the economics and the money, asthma is an expensive disease.

Dr. F. Howard Westcott.—I have recently had an opportunity to complete a study of a nineteen-year medical survey in a large commercial organization. We tried to determine the cost of various illnesses to the organization in terms of time lost by illness.

Being interested in allergic diseases, I compared the cost of asthma to the organization hiring these individuals with the cost of illness from heart disease, and a very startling figure was obtained.

The average cardiac patient losing time and figured at the rate of $7 per day, which was the average pay in this organization, cost over $800; that is, every known cardiac hired for over $500.

For every known asthmatic patient losing time it cost over $700, so that the cost to the patient is very minimum compared to the cost to organized industry.

I think Dr. Gaillard's paper is very timely, and a thing in that paper which should be emphasized now is the great need of education or we might say advertising to the laity on the importance of allergic diseases as they are manifested in early life. That lays a great burden on the shoulders of the pediatricians because this lag of ten years between the onset of the allergic manifestations and solicitation for some assistance is altogether too long.

The time elapsing between the average asthmatic or cardiac patient being hired and his solicitation of treatment shows that the cardiac patient reports to the medical department for some cardiac illness or something related to his heart sooner than four years from the time of being employed; the average asthmatic patient waits over seven years before he applies for some help for his asthma.

ABSTRACTS AND DISCUSSIONS OF TWO PAPERS READ AT THE ANNUAL MEETING OF THE SOCIETY FOR THE STUDY OF ASTHMA AND ALLIED CONDITIONS ATLANTIC CITY, N. J., MAY 2, 1942

Intrinsic Asthma: Further Observations. FRANCIS M. RACKEMANN, M.D., Boston, Mass. (Abstract)

Three years ago, in 1939, I discussed some patients whose asthma appeared to be of intrinsic origin. They fell into three principal groups.

In the first group, asthma was closely related to acute respiratory infections, and when the acute infection subsided, the asthma cleared. The patient was well until the next time which was often six months or a year later. The diagnosis of asthmatic bronchitis was descriptive.

In the second group, asthma began after the age of 30 years, but mostly after the age of 40, and then continued persistently without relation to changes in season, environment, or diet. It was not reasonable to consider that an allergy to any ordinary food or dust could play a part in the cause of trouble.

Finally, a third group included those patients who showed on physical examination evidence of emphysema, bronchiectasis, or heart disease, and who suffered from asthma because of the bronchial exudate, or edema or spasm occurring as a part of the primary disease.

Since then it became more and more certain that the conception of an intrinsic cause of asthma is entirely proper. It becomes quite possible to anticipate that in certain patients the skin tests will be quite negative to all common foods and dust substances. Whatever is the cause of asthma, it is "something which the patient carries with him." All is not allergy that causes wheezing.

The age at onset of asthma is important. Asthma which begins before the age of 30 years is extrinsic in over three-fourths (79 per cent) of the cases, but asthma which begins after 30 years is intrinsic in less than one-fourth (22 per cent) of the cases. For each successive decade the ratio of extrinsic cases becomes progressively smaller.

In December, before the Society for the Study of Asthma and Allied Conditions, Dr. Mallory preceded his lecture on the Pathology of Asthma by showing that these few patients who die of asthma are also those who begin the disease late in life.

From the Allergy Clinic and Laboratory, Massachusetts General Hospital.

Read at the Eighteenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions, May 2, 1942.

In studying the chart presented at that meeting one gets the impression that all intrinsic asthma which begins so suddenly after the age of 30 and 40 years is a progressive disease with serious prognosis.

A study of all the cases, however, modifies this impression considerably. It is true that deaths have been reported in large numbers, and I hope to study later the details concerning the cause of death and especially the age of death in the group as a whole. At the moment, however, attention is called to the considerable number of patients who are relieved of all symptoms and of other cases who are greatly improved.

One age group has been reviewed in some detail, and the plot of each ca was shown on a chart.

The fifty cases depicted have been traced through a span of twenty years in eighteen cases, and of ten years in another twenty-two cases. Fourteen patients have died: pneumonia, 3; intercurrent heart disease, 3; cor pulmonale, 1; various other diseases, 3; and, finally, asthma, 4. However, seven patients report themselves free of asthma, and eleven claim to be better. The other eighteen cases still have asthma of about the same extent as at first, whether this be mild or severe.

The clinical findings in this group are similar to what has been described in previous papers and by other writers. Paranasal sinusitis occurs in at least one-half of the cases; eosinophilia is common and positive skin tests are found in several cases, although, as said, it is not possible to ascribe any clinical significance to them. The method to which "cure" in seven patients can be attributed is interesting.

In Case 9 the onset of asthma came soon after the removal of the uterus and ovaries, and the asthma was relieved by treatment with estrogenic hormone.

The patient in Case 23, a nervous housewife, was relieved when the basis for her "nerves" was explained.

In Case 24, the patient obtained an "acid" from a friend which relieved her.

The patient in Case 26 ascribes her relief to vaccine treatment.

In Case 31 the patient has had no asthma for eleven years since an operation for peptic ulcer.

Finally two cases were relieved when abscessed teeth were found and removed.

Theories concerning intrinsic asthma are several:

1. Could it be allergy after all? There are many who think so, but unless one can postulate a reflex of the bronchial wall which has been conditioned by previous allergic episodes so that it now reacts to stimuli of different sorts, I believe that allergy can be excluded. Certainly, the attacks in this particular group of patients bear no relation to changes in season, environment, or diet.

2. A reflex mechanism from some lesion in the nose and throat is suggested by the fact that sinus disease and nasal polypi are so commonly

found in asthma. However, there are many cases without these complications and some other cause must be found for them. The term "infective asthma" is no doubt quite appropiate for a certain group, but it would hardly explain them all. When patients are relieved by treatment with hormones, with "acids," or with some more obvious form of psychotherapy, one finds it hard to consider the existence of an infectious focus which is responsible for their trouble, especially when a careful and complete physical examination is so negative.

3. One thinks of degenerative processes with such terms as primary idiopathic emphysema or pulmonary arteriolar sclerosis in the effort to explain the onset of trouble in middle life, but so far there is no direct evidence of such pathologic changes except in the occasional isolated case. One cannot recognize degenerative changes in the group as a whole.

So far, then, no theory fits quite well, and for the moment the best we can do is to keep thinking of some x factor which lies at the bottom of the problem.

SUMMARY

1. There is a type of asthma which begins after the age of 30 years, more often after the age of 40 years, in which the cause is intrinsic, something which the patient carries with him. It is a serious disease which may result in death from asthma. However, some of the patients recover and many improve. The picture as a whole is complex.

2. Theories concerning the cause are discussed.

263 Beacon Street

DISCUSSION

Dr. Abraham Colmes.—Dr. Rackemann has told us something of prognosis in asthma, a phase of which we know very little, although we have learned a good deal about the diagnosis of asthma and its treatment. When it comes to evaluating what will happen to a given individual, our knowledge is meager.

From the study of his charts, it becomes clear, even as we all have thought, but without having definite evidence for it, that the earlier the onset of asthma, the more apt is there to be a cure, the later the age of onset, the less likely is cure at all.

One fact seems important. According to Dr. Rackemann, those suffering from intractable intrinsic asthma may get better at times, and that is a point to bear in mind.

A point of issue between Dr. Rackemann and myself is on the question of intrinsic and extrinsic asthma. Evidently, from his first chart, there seems to be no definite line of demarcation between these two groups since a number of his younger patients fall into the intrinsic group, whereas some of his intrinsic patients show a tendency to remissions.

What are the criteria for the intrinsic asthma? First, the absence of positive skin reactions, but we have learned from the study of allergic diseases that extrinsic allergy may occur in the absence of positive skin reactions.

Another point is the lack of reversibility of intrinsic asthma. The mere fact that Dr. Rackemann's intrinsic patients sometimes feel better for a year or longer indicates that they are not altogether irreversible. We all have seen patients who, after the ingestion of one aspirin tablet, develop severe asthma which becomes intractable in the sense that the patients may never get entirely well. In these cases something happens to the mechanism of the bronchial apparatus; the attack, producing permanent injury of the bronchial tree, creates a state of irreversibility, and yet no one doubts that aspirin asthma is extrinsic asthma.

Infection seems present in a good many of the intrinsic asthmatic patients, but here again Dr. Rackemann stated that some of the children who were classified some years ago as intrinsic with asthmatic bronchitis, today with change in their sensitivity or a change in locality have lost their symptoms and so he has shifted them back into the extrinsic group. The occurrence of hypertension and cardiac disease and emphysema and bronchopulmonary suppurative processes in the intrinsic group suggests to me not the cause of their asthma but rather sequellae and complications which may occur in many other patients after the age of 40 years, particularly in those afflicted with asthma of the malignant type, as aptly expressed by Dr. Gay.

My idea is that in asthma of youth with onset up to the age of 40 years, the factors of allergy such as heredity and sensitization are quite apparent. The causes of such asthma are extrinsic and may come from the patient's environment, occupation, pollens, molds, drugs, cosmetics, and foods.

If the extrinsic type of patient is left untreated, the sequellae and complications are often neurosis, sinusitis, emphysema, carditis (in the broader sense), and bronchiectasis. A state of irreversibility may ensue.

In the intrinsic group, with onset of asthma after the age of 40 years, the factors of heredity and sensitization may not be quite apparent. When we search for the causes of such asthma as Dr. Rackemann always does, we find that a good many of these have neurosis, sinusitis, emphysema, heart disease, bronchiectasis, hypertension, etc. These are the very findings which constitute the sequellae and complications in the extrinsic (or atopic) patient when their asthma is left untreated.

My impression therefore is that the so-called nonsensitive intrinsic patient has been endowed with a low-grade sensitivity which remains unrecognized and which permits the disease to go on to the stage of complications and sequellae, which are no different from those of the untreated extrinsic asthma of youth.

This is a concept. It has helped me a great deal to search in the intrinsic type of patient for sensitivity not proved by skin tests. By following the patient over a long period of time and watching the variations that occur during the pollen seasons, I find after all that he is clinically pollen sensitive and possibly also sensitive to foods and environment.

Now, this is not the final word on asthma, of course, and Dr. Rackemann and I hope that the presentation of today will stir enough interest for further discussion of this very complicated and challenging phase of intrinsic and extrinsic asthma.

DR. HARRY ALEXANDER.—Dr. Colmes, if I understand him correctly, contends that intrinsic asthma is very largely a consequence of extrinsic asthma. I am sure that that is not the rule. I agree with Dr. Racke-

mann that there are cases of apparently normal individuals who have not had asthma before and who develop a cough and then wheezing and very soon have very severe asthma in whom there are at first no visceral changes whatever and who duplicate or simulate almost exactly extrinsic asthma.

If you incarcerate these people in a dust-free room and take away their food and protect them absolutely, their asthma becomes worse and not better, and thus extrinsic factors do not appear relevant.

I am so enthusiastic about Dr. Rackemann's work that perhaps what I say may be a little bit colored. I had occasion in 1938 to look up the literature on intrinsic asthma by that title and there were just two papers for the ten years previous, one of which was Dr. Rackemann's. Since then there has been very little added and Dr. Rackemann is carrying this burden pretty much alone.

Now, it is perfectly proper to say that we are allergists and this is not allergy in its true sense. However, as Dr. Rackemann tells us, if 20 per cent of asthmatic patients are in this group, then we should either take more interest in intrinsic asthma or let the internist do it. I think these cases are so important and we have done so little that I again would urge more attention and constructive effort toward solving this difficult problem.

DR. RACKEMANN.—Fortunately, Dr. Colmes and I agree about most things but fortunately too we do not agree about everything and that is the reason why we have a lot of fun in our clinic. I am always grateful to him. Dr. Colmes brings up the point that between the group which we call extrinsic and the other group which I call intrinsic there may be a close relation.

What I would like to think, and this is simply wishful thinking, is that behind both groups there is a fundamental x factor. There is something or other which enables these people to wheeze. In the one case the wheeze is started by foreign substances; by allergy in the ordinary sense; in the other case, it is started by infection perhaps or maybe by a y factor which is something which we do not know anything about. The combination of x plus y explains this intrinsic asthma.

I am very grateful to Dr. Alexander for helping me to continue to think that it is proper to conceive of asthma beginning after 40 years of age as a problem which belongs to general medicine, as having something about it that is "queer." We will keep on working that way.

Further Observations on the Treatment of Intractable Bronchial Asthma by Bilateral Resection of the Pulmonary Plexus. LESLIE N. GAY, M.D., AND WILLIAM M. RIENHOFF, M.D., Baltimore, Md. (Abstract)

In 1938 the authors published a preliminary report[1] of the therapeutic results obtained in eleven cases of intractable bronchial asthma after the posterior pulmonary plexus had been resected. This operation was undertaken after all medical therapy had failed to relieve the patients; in fact, the patients who submitted to the operation were so incapacitated

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that they had spent most of their days and nights either in the accident room or in wards of the hospital. The first report dealt with the operative technique, the postoperative course, and the subsequent clinical results. After reviewing the nerve supply to the lungs, the rationale for the undertaking was discussed. For details of the operative procedure, the authors refer you to the original publication.

Bilateral interruption is necessary to obtain what might be described as the expected physiologic result. It is well known that the mechanism involved in severe asthmatic attacks is not only a constriction of the bronchi with the consequent increase in the resistance to inflow and outflow of air in the lungs. Constriction by spasm may play an insignificant role. There is also in the patient with chronic asthma an excessive secretion from the bronchial glands. The mucus becomes tenacious and inspissated, actually plugging both large and small bronchi, and not infrequently there is edematous swelling of the mucous membranes of the small bronchioles. These findings have been confirmed by a study of the autopsy material of fifty patients who have died in the hospitals in Boston[3] and of twenty-five who have died in the Johns Hopkins Hospital.

These autopsies may be divided into several groups:

The first group included the patients who died from the effects of anoxemia and fatigue, the direct result of status asthmaticus. In this group the lungs were quite rubbery and large and did not collapse when removed from the chest.

The second group showed definite cardiac hypertrophy, usually limited to the right side of the heart, and evidence of terminal decompensation with, however, the characteristic lung changes seen in Group 1. There was little emphysema; in fact, Mallory[2] states that in these two groups emphysema was rarely encountered.

In the third group, true emphysema was encountered. The lungs were studded with areas of fibrosis, presumably the scars of previous inflammatory reaction, which surrounded minute and large areas where the air cells and respiratory bronchioles had completely ruptured.

The fourth group revealed classical vascular lesions of periarteritis nodosum, although not necessarily in the pulmonary tree or in the bronchial arteries.

With this brief review of the pathology encountered, one can readily understand the difficulties which arise when a patient is considered as a possible candidate for operation. The preoperative pathologic state will, in large measure, determine the subsequent course of events. It should be emphasized at this point that all of the patients who were treated by resection of the pulmonary plexus were critically ill with an intractable disease; medical procedures failed to give even transient relief. Without resort to drastic treatment, the outcome of the disease was as obvious as in any case of advanced malignant disease.

OPERATIVE RESULTS

The twenty-one patients treated in this clinic by resection of the pulmonary plexus may be divided into three groups for analysis.

In Group 1, the successfully treated patients, the age at the time of operation varied from 25 to 54 years. The duration of the symptoms had been from eight months to twenty years. The interval of time elapsing between the first and second operations varied from eight to twenty-eight days. Three of these patients were operated upon eight years previously, two 7 years previously, one 5 years previously, and the seventh patient has been free from severe asthma for the preceding four years. Unfortunately, the oldest patient, 54 years of age, died eight months after operation.

In Group 2 there were nine failures. The youngest of these patients was 30 years of age, the oldest was 54. All had suffered with asthma for from six months to twenty-two years. Three of the group died following the first operation; the causes of death were intrapulmonary hemorrhage, pneumonia with cardiac collapse, and postoperative empyema with subsequent coronary occlusion. Three patients died following the second operation, from acute pulmonary embolism, acute gastric distention, and acute intrapulmonary hemorrhage, respectively. Of this group of six patients, two died on the operating table and four died six to seven days after operation.

The four patients in Group 3 should not have been subjected to this radical procedure. These patients had been carefully studied in the Henry Phipps Psychiatric Clinic, and although they showed the classical signs and symptoms of violent status asthmaticus, their attacks invariably followed emotional disturbances or mental strain.

SUMMARY

1. Twenty-one patients have been treated in this clinic by bilateral resection of the pulmonary plexus. Of these, eight derived striking benefit, nine died as a result of, or subsequent to, the operation, and four who failed to improve following operation are still suffering with severe bronchial asthma.

2. The twenty-one patients had suffered with violent attacks of asthma in periods varying from one to twenty-eight years. The duration of the disease had no apparent effect upon the therapeutic success of the operative procedure. One patient, 39 years of age, had suffered with asthma for twenty years; since the operation eight years ago she has never had an attack of asthma. The ages of the patients seemed to have no relation to the outcome; the youngest was 25 years of age and the oldest was 55.

3. In every instance, these patients suffered with asthma of intrinsic type, the result of chronic respiratory infection and the subsequent infection of the pulmonary system. Allergy from extrinsic factors apparently played little part in the production of the asthmatic paroxysms.

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DISCUSSION

DR. LOUIS TUFT.—I believe that we all are indebted to Drs. Gay and Rienhoff for their paper on the surgical treatment of intractable asthma. In such a recalcitrant condition in which new remedies constantly are being proposed, it is essential that each new therapeutic procedure be given a fair trial to permit of proper evaluation. That is what has been done for us by Drs. Gay and Rienhoff. They have given us the results of an experience unparalled by any other clinic or group of workers. As a consequence we should now be in a position to decide whether this procedure should be added to our therapeutic armamentarium and to what type of case it is best adapted. That it is a radical and risky procedure has been duly emphasized; likewise, the chances of success are not too great. Thus, from the results obtained in Groups 1 and 2, comprising the only cases where operation was indicated, one could say to a patient about to have this operation that he had about a 70 per cent chance of surviving the operation, and if he survived, there was only a 50 per cent chance of any degree of success.

The sole encouraging note of the report is the fact that complete and prolonged relief of symptoms for as long as eight years occurred in three of the seventeen cases. This is not a large percentage, considering the gravity of the procedure. It indicates, nevertheless, that this treatment may have a place in a limited group of patients, provided that the exact indications for its use are more clearly defined.

Drs. Gay and Rienhoff state clearly that it is to be used in those chronic asthmatic patients in whom no other therapeutic method is likely to give relief. However, I would like to ask them, for the sake of clarification, how they determined this, particularly in those cases included in their report who were comparatively young and had asthma for relatively short periods of time, from six months to one to two years. I think that it is important for us to know, for example, what the reasons were for operating on the patient in Case 3 in Group 1 who had asthma for only eight months and on the patient in Case 1 in Group 2 with asthma of six months' duration.

In a condition as capricious as asthma, even when it seems to be intractable a great deal of courage is required to recommend so radical and dangerous a procedure in patients with asthma of short duration. For that reason I have hesitated in recommending its use in the past. In preparing this discussion after reading over a copy of the present paper, I tried to reflect back over the many intractable cases of asthma that we have had, to see in which ones I would now have recommended surgery instead of such measures as fever or x-ray therapy, climatic change, etc.

Although many of them entered the hospital in status asthmaticus and appeared rather hopeless at the time, it is surprising what the final out-

come was in some of them after continued treatment. As nearly as I can judge from their present situation and follow-up reports, it seems to me that they have fared as well generally and in some instances better than the cases reported here today and certainly with much less attendant risk. I can think, at the moment, of one individual in whom in the light of subsequent events and of our present knowledge, surgery would have been indicated. This was a woman about 40 years of age with a bacterial type of asthma of eighteen years' duration who took fifteen to twenty injections of adrenalin daily for nine years and who died in a severe asthmatic paroxysm three years after she left the hospital. She represents the type of case, in my opinion, in which surgery would have been indicated.

It is true that patients with long-standing asthma are not suitable surgical risks and do not represent ideal subjects; nevertheless, considering the risks to be taken and the possible chances of success, I believe that for the present bilateral resection of the pulmonary plexus should be used as a last resort and only in patients with intractable asthma of long duration who require almost constant medical attention. I think that we would all appreciate if Drs. Gay and Rienhoff would define for us more specifically the type of patient in whom, in the light of their present experience, they would advocate this treatment, so that we might be properly guided in the future.

Dr. Horace Baldwin.—I feel indebted to Dr. Gay for a summary of the follow-up of these cases. On the other hand, from just hearing his paper, I feel that it is a method where the risk is so great and where the rationale is so limited that the tenor of the paper should be distinctly condemnatory of the procedure rather than leaving very much of a loophole to allow us to feel that it offers very much hope.

After all, there are three of twenty-one patients who had very definite relief. I must say that I am entirely in accord with Dr. Tuft and I agree with what he said, that that percentage of patients might well have secured that amount of relief with other, conservative measures that we employ.

I should certainly hate to see this method generally applied and it is certainly my hope that the paper will not add much encouragement to the performance of this procedure in many, if any, asthmatic patients of the status asthmaticus type.

Dr. Robert A. Cooke.—I merely wanted to ask a question of Dr. Gay. It was not quite clear how he differentiated between Groups 1 and 2. I understood Group 3, yet I did not get the differentiation in Group 1, where the results were satisfactory and in Group 2, where they were very unsatisfactory. It certainly was not on the matter of age or duration of the trouble.

Dr. Gay.—I am afraid that Dr. Baldwin did not understand the purpose of my paper. I specifically stated that the paper is presented as an answer to the many inquiries from the members of this Society as to the final opinion of the surgical procedure.

I do not urge it as a means of treatment of the asthmatic patient and wish to emphasize further that there are no criteria as yet established

which assist us in arriving at a decision as to when or not to operate. It should be kept in mind in those cases of asthma which assume a malignant form.

Now, with regard to Dr. Cooke's question concerning Groups 1 and 2, all patients were suffering from asthma of an intrinsic type. Group 1 consisted of patients who recovered from the operation, while Group 2 included the patients who derived no benefit and who died from and subsequent to the operation.

Dr. Tuft asks the question why Case 2 in Group 1 was subjected to operation, having had asthma for only eight months. This man had been thoroughly studied and found to have an intractable type of asthma. He was one of our earliest cases, having been operated on seven years ago. He was in good physical condition but completely incapacitated and because of our excellent result in the first case, we felt justified in treating Case 2 by surgery.

It should be emphasized that the procedure outlined in the original paper and supplemented by this report should not be carried out unless a competent lung surgeon is available. I have been very fortunate in having Dr. Rienhoff in Baltimore. He is recognized as one of the outstanding lung surgeons in America today.

In conclusion I might say that the eight patients presented themselves before the Johns Hopkins Medical Society several months ago, having previously been completely incapacitated. It was very gratifying to see them, entirely free from discomfort, each one now having a lucrative occupation.

Selected Abstracts

Immunology

UNDER THE DIRECTION OF MATTHEW WALZER, M.D., AND
MURRAY ALBERT, M.D., BROOKLYN

Studies on Histamine Sensitivity and Anaphylactic Response. The Effect of Thyroid Extract. Farmer, L., and Fribourg, R.: Proc. Soc. Exper. Biol. & Med. **50**: 208, 1942.

The effect of the administration of thyroid extract upon the histamine and anaphylactic responses in guinea pigs was investigated. The administration of five doses of thyroid extract over a period of five to six days increased the susceptibility of the animals to histamine shock. This increased reactivity to histamine following thyroid administration is offered as an explanation for the enhanced anaphylactic response observed by previous investigators working with thyroid. It is suggested that thyroid may deplete the ascorbic acid and cholesterol stores of the adrenals, and this may lead to depletion of their cortin content. This in turn produces an increase in histamine sensitivity and enhances the anaphylactic response. In an effort to prove this hypothesis, ascorbic acid determinations were made on the adrenals of normal guinea pigs and of animals which had been fed thyroid. In the latter group the readings were much lower than in the normals.

Bovine Albumin as an Antigen. Taylor, H. L., and Keys, A.: Proc. Soc. Exper. Biol. & Med. **50**: 325, 1942.

The behavior of bovine albumin as an antigen was investigated because of its suggested use in man. It was prepared by fractionation of bovine plasma with ammonium sulfate. Sensitized rabbits showed antibody titers up to 1:24,000. Guinea pigs, sensitized with bovine albumin, could be shocked with a single intracardial injection of the antigen. The optimal shocking period was eighteen to twenty-one days after the last injection. A history of a case in which bovine albumin acted as an antigen in man is cited. The patient, who had shown no skin reaction to albumin, was given a single intravenous injection of bovine albumin. Five weeks later the skin test was positive. Intravenous injection of the antigen resulted in a marked reaction consisting of cyanosis and temporary cessation of respiration, indicating that bovine albumin is a potent antigen.

A Quantitative Study of Anaphylaxis in the Guinea Pig. Kabat, E. A., and Landow, H.: J. Immunol. **43**: 69, 1942.

The amounts of antigen and antibody involved in the production of anaphylaxis in guinea pigs were investigated. The sera of rabbits, actively sensitized with crystallized egg albumin or type III pneumococcal polysaccharide, were used to produce passive anaphylaxis in the guinea pigs. It was found that approximately 0.03 mg. of antibody nitrogen was sufficient to sensitize the animals, so that twenty-four hours later an injection of 1 mg. of egg albumin resulted in uniformly fatal anaphylactic shock. The animals passively sensitized with pneumococcal antisera required

0.1 mg. of polysaccharide as a shocking dose. Sensitization with 0.006 to 0.025 mg. of antibody nitrogen produced anaphylactic symptoms irregularly, and smaller amounts of antibody did not sensitize at all. The greatest number of fatal reactions occurred when an excess amount of antigen was injected (1 mg. or more) as a shocking dose. With 0.1 mg., the number of fatalities was diminished. It was found that the uteri of pigs sensitized intravenously with 0.03 mg. of antibody nitrogen invariably contracted when 1 mg. of antigen was added to the Dale baths. When smaller amounts of antibody nitrogen were used for sensitization, contractions of the uteri could not be elicited regularly. The sensitivity of the isolated uterus was of the same degree as that of the intact animal. Assuming that the injected antibody is distributed uniformly throughout, it was computed that the uterus need contain only 0.01 mg. of antibody nitrogen in order to contract. It was apparent from these experiments that the ordinary serologic tests, in many instances, were not sensitive enough to detect the presence of antibody in the blood and tissues of actively sensitized guinea pigs. Similarly, in human beings, precipitins, agglutinins, and complement-fixing antibodies cannot be found with the ordinary methods in human sera which contain demonstrable skin-sensitizing antibodies. If the affinity of the skin for the skin-sensitizing antibody is about the same as that of the guinea pig uterine muscle for the anaphylactic antibody, then only 0.01 mg. of antibody nitrogen would be required to sensitize a passive transfer site. The mechanism of desensitization in anaphylaxis is discussed.

Immunological Studies of Pollinosis. III. Fluctuations in Antibody Titers of Normal Individuals Subcutaneously and Intravenously Injected With Pollen Extract Over Protracted Periods. Loveless, M. H.: J. Immunol. **43**: 1, 1942.

Three normal individuals were injected subcutaneously and intravenously with saline and alum-precipitated extracts of low ragweed pollen over protracted periods of time. Their sera were titrated at various intervals for thermostable neutralizing antibody. The effect of the reinjection of pollen antigen as a secondary stimulus after a protracted rest period was also investigated. The immune antibody content of the sera was estimated by measuring its antigen-inactivating power. Individuals who received injections of excessive amounts of ragweed pollen, twice weekly for several months, showed the presence of immune antibody in their sera. The degree of immune response varied with the individual. Two subjects receiving similar dosages of ragweed pollen extract showed a marked difference in immune response. Small doses of alum-precipitated pollen antigen produced a greater antibody response than larger doses of plain pollen antigen. A marked enhancement of the immune response occurred following the subcutaneous injection of pollen antigen after a rest period of three or more months had elapsed after the initial course of pollen injections. When the secondary stimulating doses were administered intravenously, the immune response was not as great.

None of the three subjects showed any reagins against ragweed antigen following protracted periods of treatment with pollen extracts. One individual developed a temporary cutaneous sensitivity, but this was not associated with clinical symptoms. Mild local reactions were observed after subcutaneous injections of either plain or alum-precipitated extracts.

Correspondence

The Editor,

THE JOURNAL OF ALLERGY.

I would like to correct and amend the data on the allergy clinic of the University of Virginia as published in the report of the Committee on Postgraduate Education in Allergy in the July issue of the JOURNAL. The correction is that my clinic was approved by the Advisory Board of American Allergists in December, 1941. The first amendment is that the regular staff of the clinic consists of a fellow in allergy, an assistant resident, and an intern in medicine.

The second amendment is that we have a fellowship in allergy and arthritis which was established on July 1. Very little will be done in arthritis until after the war, as our plans for starting an operative clinic had to be postponed until then.

Applications for the fellowship will be accepted at any time.

OSCAR SWINEFORD, JR.

University, Va., Aug. 4, 1942.

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